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**Introduction to
AGRICULTURAL
BIOCHEMISTRY**

Introduction to AGRICULTURAL BIOCHEMISTRY

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2002

Introduction to

PRINTED IN THE UNITED STATES OF AMERICA

To the memory of
DR. DENNIS EDWARD HALEY
(1885-1945)

*Beloved Teacher, Ardent Investigator
Devoted Student Counselor
and Respected Colleague*

Preface

*Science moves, but slowly, slowly,
creeping on from point to point.*

TENNYSON

The gradual accumulation of knowledge during recent years has resulted in a considerable modification of our conceptions regarding the mechanism of physical and chemical changes involved in life processes. Our ideas concerning the chemistry of plant and animal life have undergone appreciable revision since the publication of *Introduction to Agricultural Biochemistry* (Dutcher and Haley) in 1932. Although the present volume follows the general plan outlined in the previous textbook, so far as subject matter is concerned, it is in reality a new book, since all chapters, with the exception of Chapter 1, have been completely revised and rewritten. Chapter 1 has been enlarged to include data on the history of the development of animal biochemistry (physiological chemistry) in Europe and America.

Part 1 is designed to stimulate interest by introducing the student to some of the interesting and significant reasons for the study of agricultural biochemistry, to review the organic chemistry of compounds of biological importance, and to introduce definitions, terms, and mechanisms which will help the student understand and appreciate material presented in subsequent chapters.

Part 2 (The Plant) involves a discussion of the more important chemical facts and theories relating to plant growth, from the time the seed germinates until it becomes a mature plant. The chapter on farm chemurgy is designed to acquaint the student with actual and potential utilization of farm crops for industrial purposes. So far as the authors are aware, this is the first textbook in agricultural biochemistry to discuss the chemical aspects of farm chemurgy in a comprehensive manner.

Part 3 (The Animal) has been written with the view of stressing, so far as possible, the biochemical phases of metabolism and growth. Practical applications have not been stressed since this can be done to better advantage in subsequent practical courses dealing with livestock feeds and feeding. Tables of recommended nutrient allowances for humans and domestic animals and tables of chemical composition of some selected human foods and livestock feeds have been placed in the appendix for reference purposes.

The book has been written on the assumption that it will be suitable for students with sound training in inorganic and organic chemistry. It is hoped that the present volume will stimulate interest in the teaching of agricultural biochemistry and that it will also serve as a general reference book for students who are interested in the underlying chemical principles affecting plant and animal growth.

We wish to acknowledge the helpful counsel and assistance of Professors D. E. H. Frear, A. C. Richer, and R. W. Swift in reading and criticizing certain portions of the manuscript. We are also indebted to Anita Zellers and Frances Sowko for assistance in preparing the manuscript for publication.

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January 1951

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Part I.
General and Introductory

I · The Development of Agricultural Chemistry

When we attempt to study the factors that have played important roles in the development of scientific agriculture, we find that chemistry has occupied a most prominent place. The part that chemistry has played in this development has been of such far-reaching importance that a special branch of this science, known as agricultural chemistry, has been a natural outgrowth. It is to this particular phase of chemistry that we wish to direct the reader's attention, for agricultural chemistry, probably more than any other single factor, has been responsible for the development of the quantitative aspects of modern agricultural practice and for the elimination of the old "rule-of-thumb" methods which had been followed for centuries.

THE INFLUENCE OF ALCHEMY

If, in turn, we wish to trace the historical factors that had to do with the development of the science of chemistry, we must go back to the very beginning of chemical effort, namely, to the beginning of alchemy. It has been suggested that the name alchemy is of Arabic origin, probably dating from the eighth century. In the Arabic the prefix *al* means *the*, and *chemia* is the equivalent of *to hide*. Some authorities believe that the word alchemy is derived from the Egyptian word *chemi*, meaning *black*. At least it is clear that the early workers looked upon alchemy as the hidden art or science, and in fact for centuries it was considered the black art.

The writings of the very early alchemists have been lost, but those of a later date which have been deciphered are replete

with mysticism and characterized by obscure terminology, accurately described by a recent writer as “incomprehensible nonsense.” It is quite evident that many of these early alchemists were far from honest. All of them had but three goals toward which they were striving: the first, the transmutation of gold



FIG. 1. “The Alchemist.” From a painting by David Teniers the Younger.
(Courtesy of the Fisher Scientific Company.)

from the baser metals; the second, to find or discover the “philosopher’s stone,” the possession of which would permit the owner to perform miraculous feats, such as transmutation, the extension of human life, curing of disease, etc.; and the third, the discovery of the “elixir of life,” which would ensure perpetual youth. In spite of the fact that alchemy, by even the greatest flight of fancy, could not be termed a science, nevertheless the science of chemistry as we know it today owes its origin to the labors of the alchemist.

It is clear that Aristotle, in the fourth century B.C., undoubt-

edly gave impetus to the alchemical concept of the structure of matter which had been the result of philosophical thinking since the fifteenth century B.C. This postulation conceived the universe to be made of the four elements, earth, air, fire, and water. As the art of alchemy developed, these terms gradually became somewhat comprehensive in nature. All vapors, gases, steam, and smoke were included under "air," and sparks, lightning, and all incandescent objects were described as "fire." Likewise all liquids, such as blood, milk, and chemical solutions, became known as "water," and all solids were called "earth." As a result, we have today the term "rare earth metals."

These early workers knew that water could be converted into steam and that steam could be condensed into water. What could be more natural, therefore, than to conceive that metals could be transmuted from one form to another?

In addition to the term "rare earth," mentioned in a preceding paragraph, we have also retained the alchemical terms alcohol, borax, alkali, and elixir. Many of the alchemical writers refer to Hermes Trismegistus as the Father of Alchemy, and for this reason the "hidden art" has become known as the "hermetic art." It is from this source that we get our modern term "hermetically sealed." Geber, an Arabian physician, attained considerable fame as an alchemist, and to him is ascribed the credit of having written the first chemical textbook, entitled *Summa Perfectionis* (*The Summit [or Height] of Perfection*). Paracelsus, in the sixteenth century, was the first to call attention to the interdependence of chemistry and medicine. The union of chemistry and medicine was a fortunate circumstance for both, but particularly for the science of chemistry, for the reason that it attracted men of culture and education. As a result the new workers in the medical-alchemical field discovered new compounds and paved the way for the development of organic chemistry. Another and equally important situation developed as a result of the interest displayed by the physicians of that time. Prior to this period the alchemist had been striving purely for selfish purposes, for what he could gain personally by obtaining a quick and easy way to riches and perpetual youth. The new workers, like the research workers of today, began to seek for new knowledge, not so much for economic gain, but

rather for the general good of mankind and for the intellectual satisfaction derived from constructive, purposeful, and unselfish labor.

THE BEGINNING OF GENUINE CHEMISTRY

The alchemical period which we have described so briefly comprised, roughly, the period 300 B.C. to 1700 A.D. We shall point out but one matter of consequence characteristic of the seventeenth century. It was during this period that the phlogiston theory was advanced; in fact, the seventeenth century might well be called the phlogiston period of chemical development. This theory was advanced to explain the phenomenon of combustion. The advocates of this theory contended that a substance to be combustible must contain a substance known as phlogiston. Reasoning in that manner, they concluded that wood or coal, for example, contained large quantities of phlogiston which escaped during combustion, whereas incombustible stones or iron were practically devoid of this important substance. It was not until the discovery of oxygen that the true nature of combustion was demonstrated.

Genuine chemistry had its beginning during the eighteenth century, with the work of Priestley, Cavendish, Scheele, and Lavoisier. The work of Priestley is important from a historical standpoint for his isolation of oxygen and the study of oxidation. Cavendish enriched our early knowledge through the use of the electric spark in the formation of nitric acid from the oxygen and nitrogen of the air and water. Scheele, a Swedish chemist, contributed to the chemistry of oxygen and chlorine, and his researches gave us, for the first time, bleaching powder and chloroform.

Up to this time the phlogiston theory was quite generally accepted. It was not until the work of Lavoisier, a French chemist, that the true nature of oxidation was demonstrated. All these workers and many others exerted a definite influence on the development of true chemistry and on the development of modern agricultural chemistry.

SEARCH FOR THE "PRINCIPLE OF VEGETATION"

When we turn our attention to the early workers who were interested in solving nature's secrets as they relate to agriculture, we find nearly all of them trying to discover "the principle of vegetation." In other words, they were seeking to answer the question, "Why and by what method do plants grow and develop?" Of course, these observers were no less observant than the nature lover of today. As a result they had noted that plants often grew well in certain soils although the same crops did not yield well in other soils, even under similar climatic conditions.

One of the first theories which aimed to explain the secret of plant growth was that advanced by a Belgian physician and alchemist by the name of van Helmont. This investigator, working in the latter part of the sixteenth and the early part of the seventeenth centuries, was among the first to introduce the use of the balance and to interpret data from the quantitative standpoint. It should be remembered that water was one of the recognized chemical elements at the time of van Helmont's work, and as a result of his studies he concluded that water must be the "principle of vegetation," for he cited the following experiment as proof of his theory that water could be transformed into plant tissue. Using van Helmont's own words: "I took an earthen vessel in which I put two hundred pounds of soil, dried it in an oven, then I moistened it with rain water, and pressed hard into it a shoot of a willow weighing five pounds. After exactly five years the tree that had grown up weighed one hundred sixty-nine pounds and about three ounces. But the vessel had never received anything but rain water or distilled water, to moisten the soil when this was necessary, and it remained full of soil which was tightly packed, and lest any dust from the outside should get into the soil, it was covered with a sheet of iron coated with tin, but perforated with many holes. I did not take the weight of the leaves that fell in the autumn. In the end I dried the soil once more and got the same two hundred pounds that I started with, less about two ounces.

Therefore, the one hundred sixty-four pounds of wood, bark and roots arose from the water alone."

This experiment, however, is thoroughly typical of much of the early investigational work in agricultural chemistry, as well as of other sciences. In this, as in other branches of science, it is very easy to fail to consider a vital factor and, as a result, draw, from perfectly good experiments, a conclusion which appears to be correct but which is, in reality, entirely wrong. In the work cited above, van Helmont failed to take into consideration two most important factors, namely, the role played by the constituents of the atmosphere, and the two ounces of soil which had disappeared. Hopkins has very truthfully said that "An experiment is a question put to Nature, and Nature always answers every question truthfully, but the question that Nature answers and that the experimenter asks is not always the question that he thinks he asks."

Some years after van Helmont reported his results, Glauber proposed the hypothesis that saltpeter is really the "principle of vegetation." This conclusion was reached by Glauber because he secured such large increases in the yield of crops by applying this material as a fertilizer. In support of his conclusion he also cited the fact that he "obtained saltpeter from the earth cleared out from cattle sheds," and that "it must have come from the urine or droppings of animals and must, therefore, be contained in the animal's food, i.e., in plants." For many years this view of Glauber was widely accepted by agricultural writers. The only prominent opponent to this view was Jethro Tull, who believed that the fineness of the soil particles had a beneficial influence on plant growth. According to his view it "was the very minute particles of soil loosened by the action of moisture that constituted the proper 'pabulum' of plants. The pressure caused by the swelling of the growing roots forced these particles into the lacteal mouths of roots where they entered the circulatory system. All plants live on these particles, that is, on the same kind of food." Various other ideas regarding the "principle of vegetation" were proposed. The general view held at the close of this period cannot be better summed up than in Tull's own words: "It is agreed that all the following materials contribute in some manner to the increase of plants, but it is

disputed which of them contributes most to that increase of food: nitre, water, air, fire, and earth."

During the latter half of the eighteenth century a considerable interest was manifested in all phases of agriculture. Textbooks were written, experimental work was stimulated, and societies were formed for the promotion of agriculture. In 1755 the Edinburgh Society of England employed a chemist by the name of Francis Home "to try how far chemistry will go in settling the principles of agriculture." Home, believing that the whole system of agriculture was dependent upon plant growth, prosecuted his research along the lines of plant nutrition, finally drawing the conclusion that there were at least six plant food materials: air, water, earth, salts of different kinds, oil, and "fire in a fixed state."

After the work of Home there was no important advance in agricultural chemistry for forty years. From what has been stated, it is evident that anything like an adequate idea of the growth and composition of plant bodies could not be obtained until certain of the important chemical elements had been discovered and the composition of water and other common substances had been established.

During the period 1770 to 1800 some of this necessary work was accomplished, but its importance in agriculture was not appreciated at the time. This work, the discovery of oxygen by Priestley and by Scheele, the discovery of the composition of water by Cavendish, and the explanation of combustion by Lavoisier has already been cited. These discoveries served to overthrow alchemy and opened the way for the development of modern chemistry.

THE BEGINNING OF MODERN AGRICULTURAL SCIENCE

After the work of Home further progress in scientific agriculture was hardly possible until greater use was made of accurate chemical methods of investigation. This type of experimentation soon found expression in the epoch-marking work of the French chemist, Théodore de Saussure. To this investigator is due much of the credit for the quantitative statistical method

which has been the basis of all scientific work since that time.

Although Ingen-Housz receives the credit for the discovery of the role that carbon dioxide plays in plant economy, it remained for de Saussure to place this discovery on a firm scientific basis by the use of quantitative methods. His book, *Researches upon Vegetation*, published in 1804, was really the first scientific work showing the source of the carbon compounds in plants. He established, by means of quantitative experiments, that the increase in the amounts of carbon, hydrogen and oxygen, when plants are exposed to the sunlight, was obtained from the carbon dioxide of the atmosphere and the water of the soil. This early investigator also stated that the mineral elements derived from the soil are essential to plant growth; in proof of this point he gave the results obtained from the analyses of the ash of many different plants. He believed that plants obtain the greater part of their nitrogen from the soil. These views of de Saussure have since been investigated and verified by many different scientists and are substantially those held at the present time regarding the fundamental principles of plant nutrition. In the days of de Saussure, however, this view was not accepted as true, and it was nearly half a century later that Boussingault, Liebig, and others repeated the investigations of de Saussure and confirmed his results, which were finally accepted by the scientific world.

From the time of de Saussure's work until 1835 there was little active work in progress relating to agricultural science, but the facts which had already been accumulated were given attention and some attempts were made to apply the results to actual practice. Between 1802 and 1812 Sir Humphry Davy delivered lectures annually on agricultural chemistry. These lectures were published in book form in 1813 and entitled *Essentials of Agricultural Chemistry*. This was the first textbook of the modern period, and it treated of the composition of the air, soil, manure, and plants, and of the influence of heat and light on plant growth. Although some of the views expressed in this work were erroneous, it was for the most part a carefully prepared summary of the best accepted knowledge obtained from the results of previous investigations. One of Davy's contemporaries, Thaer, also published (1809-1810) an

important work entitled *Principles of Rational Agriculture*. Thaer proposed the so-called humus theory of soil fertility. He believed that plants obtain their nourishment from the humus, and consequently it is this material which determines the productivity of the soil. This idea of Thaer's, however, was shown later to be inadequate in accounting for the sources of plant food, and as a result it prevented, for a time, the recognition of the actual value of humus as a factor in soil fertility. Although some of Thaer's conclusions were erroneous, his writings were of a very practical nature and did much to stimulate investigational work.

About 1830 there was a revival of interest in scientific investigations which related to agriculture. At this time Boussingault, a Frenchman, became actively engaged in agricultural research and began a series of field experiments on his farm in Alsace. He was the first investigator to have a chemical laboratory on a farm and to make thoroughly practical investigations in connection with agriculture. In fact, the establishment of this laboratory marked the start of the first agricultural experiment station, although Mary Louise Foster in her *Life of Lavoisier* states that, through the efforts of Lavoisier, the French Government established an experimental farm in Villefrancoeur as early as 1778.

Russell states that Boussingault "reintroduced the quantitative methods of de Saussure, weighed and analyzed the manures used and the crops obtained, and, at the end of the rotation, drew up a balance sheet, showing how far the manure had satisfied the needs of the crops and how far other sources of supply—air, rain, and soil—had been drawn upon." He also did very important work upon the assimilation of the free nitrogen of the air by plants. He ascertained many important facts relating to the chemical characteristics of foods, made a comparison as to the quantity of nitrogen in different kinds of feeding stuffs, and compared their values on the basis of the nitrogen content. His study on the production of saltpeter did much to prepare the way for later work on nitrification. Russell summarized the work of this scientist in the following words: "Boussingault's work covers the whole range of agriculture and deals with the composition of crops at different stages of growth, with the soil,

and with the problems in animal nutrition." Much of the earlier work of de Saussure was repeated and verified by Boussingault; during this work many additional facts regarding the chemistry of plant growth were ascertained.

Regarding the source of nitrogen in farm crops he states, "The soil furnishes the crops with mineral alkaline substances, provides them with nitrogen, by ammonia and nitrates, which are in the diluvium which is the basis of vegetable earth, compounds in which nitrogen exists in stable combination only becoming fertilizing by the effects of time." As to the absorption of gaseous nitrogen of the air by vegetable earth, Boussingault also states, "I am not acquainted with a single irreproachable observation that establishes this. Not only does the earth not absorb gaseous nitrogen, but it gives it off."

No discoveries of importance are recorded during the period 1830 to 1840. In 1840, however, Justus von Liebig's report to the British Association (published later as *Chemistry in Its Application to Agriculture and Physiology*) created a great amount of discussion in scientific circles. Liebig was a very forceful writer and seemed to delight in scientific polemical discussions. He berated the plant physiologists for their lack of chemical knowledge and took them to task for ignoring the accumulating experimental evidence that plants receive their carbon supply from the atmosphere rather than from the carbonic acid of the soil. He stated, "All explanations of chemists must remain without fruit and useless because, even to the great leaders in physiology, carbonic acid, ammonia, acids and bases are sounds without meaning, words without sense, terms of an unknown language, which awake no thought and no association."

Prior to this time many workers adhered to the humus theory of Thaer, which postulated that it was possible for the plant to satisfy its carbon demands entirely from humus or soil organic matter. The publications and lectures of Boussingault and de Saussure had made little impression on the proponents of the humus theory. After Liebig's tirade, however, there were few who dared to oppose his theory of carbon dioxide assimilation. In the first edition of his book Liebig called attention to the fact that farms from which certain products were sold gradually became less productive because of the decrease of nitrogen, but

he made one serious mistake. He overestimated the amount of ammonia present in the atmosphere and underestimated the value of nitrogen in the soils and manures. A study of the chemical composition of the ash of plants was also made by Liebig. His results led him to propose the mineral theory of

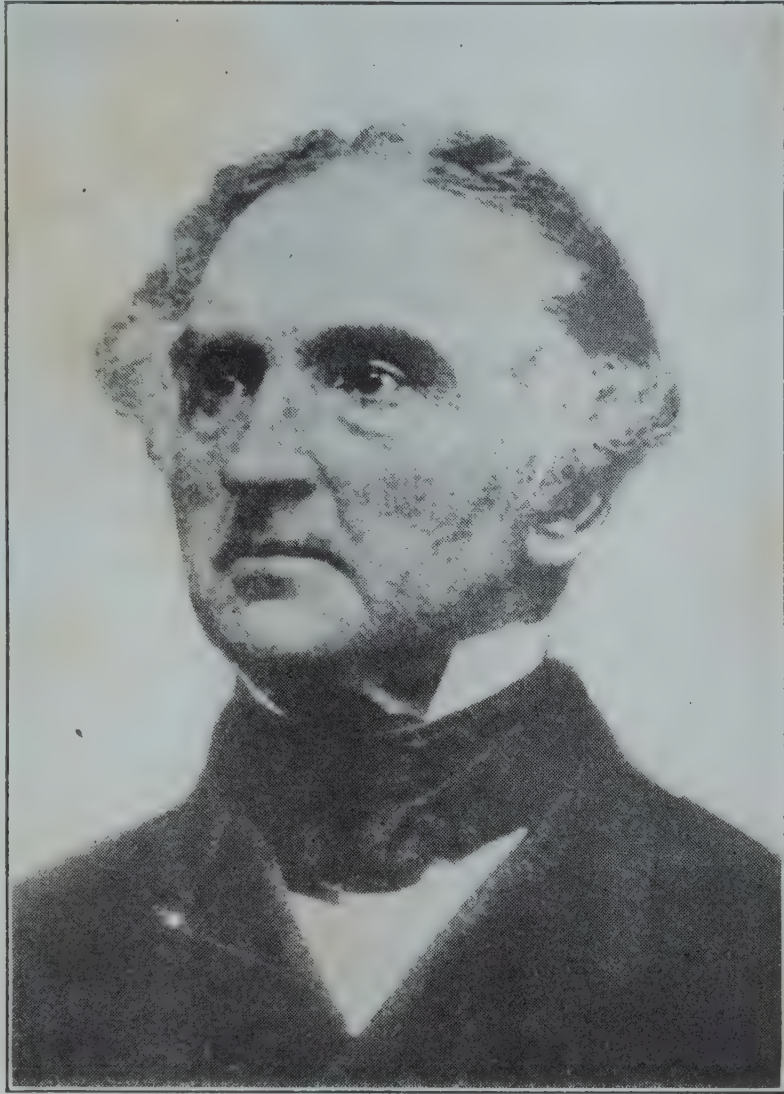


FIG. 2. Justus von Liebig (1803–1873), sometimes called the Father of Agricultural Chemistry. (Courtesy of McGraw-Hill.)

plant nutrition. Previous to this time de Saussure had proved that plants contained certain mineral elements, but he had laid no emphasis upon their importance as plant foods. Liebig's publications on the composition of the mineral substances present in plants, and his emphasis of their importance as plant foods, led to the commercial preparation of manures which in later years has developed into the great commercial fertilizer industry.

Liebig's work was unlike that of Boussingault in that it was not conducted in connection with field experiments. It had,

nevertheless, a very stimulating influence upon researches in agricultural chemistry, and to him we owe, to a large extent, the collecting and summarizing of the previous work and the pointing out of valuable lines for future investigation. Liebig's great enthusiasm for agricultural investigation may be judged from the following extract: "I shall be happy if I succeed in attracting the attention of men of science to subjects which will merit engaging their talents and energies. Perfect agriculture is the true foundation of trade and industry. It is the foundation of the riches of states, but the rational system of agriculture cannot be formed without the application of scientific principles, for such a system must be based on an exact acquaintance with the needs of the nutrition of vegetables, and with the influence of soils and action of manures upon them. This knowledge must be based on chemistry, which teaches the mode of investigating the composition, and of the study of the character of the different substances from which plants derive their nourishment."

Liebig was a man of personality. He gathered around him a large following of students, many of whom later became prominent in scientific work. He is sometimes called the Father of Agricultural Chemistry. Doubtless it will be of interest to students to know that, though the name of Liebig will go down in history as that of a man of great learning and genius, as a boy in school he was a "sorrow to his parents and a burden to his teachers." It is said that he had no "ear memory," and it was finally necessary to take him from school, at which time his father apprenticed him to an apothecary. In the course of his experimenting he blew the window out of the shop and was dismissed. By hard study in libraries and in the private laboratory in his home he obtained such proficiency that he was able to enter the university, and at the early age of nineteen he obtained the degree of doctor of philosophy. He attracted so much attention that he was given permission to work in the laboratory of the famous Gay-Lussac. These two workers made an important discovery relating to the chemical and physical properties (isomerism) of certain organic compounds. When they obtained the final proof that they had made the discovery they wrapped their arms about each other and waltzed around the laboratory. This personal reference is made here to show

something of the ardor with which Liebig went about his work.

Shortly after Liebig's first work appeared the investigations at Rothamsted, England, which were begun by John Lawes, a young Englishman who at an early age became interested in chemical investigations. When he was a boy, experiments made at home seem to have been one of his favorite occupations. He tells us: "At the age of twenty, I gave an order to a London firm to fit up a complete laboratory, and I am afraid it sadly disturbed the peace of my mother to see one of the best rooms of the house fitted up with stoves, retorts, and all the apparatus and reagents necessary for chemical investigation." In 1837, on his home farm at Rothamsted, he commenced experiments, in pots, with agricultural plants and the application of various manures. Lawes was much interested in the results he obtained and later continued the experiments on a larger scale. He soon found that to carry on his work in a proper manner he needed the assistance of a trained chemist, and he engaged the services of Dr. J. H. Gilbert, one of Liebig's former students, who began his work at Rothamsted in 1843. This marked the establishment of the second agricultural experiment station; it is still in operation at the present time, having been endowed with funds by John Lawes. Many of the Rothamsted experiments have been conducted continuously since 1844, and results of the greatest value to agriculture have been obtained as a reward of the earnest, persistent work of Lawes and Gilbert.

Lawes was the more practical type of man and directed the agricultural operations on the experimental plots. The execution of the remainder of the experimental work was largely in the hands of Dr. Gilbert, who was a thoroughly trained chemist. Dr. A. D. Hall, one of the recent directors of the Rothamsted station, says of Gilbert:

His special mental characteristics also eminently fitted him for the work subsequently carried out. He was both cautious and painstaking to a remarkable extent, desiring to accumulate a great mass of facts before coming to any certain conclusion upon them. His mode of work was also extremely methodical and a method once adopted after full consideration was continued through many subsequent years, thus giving rise to a long series of results obtained in a perfectly similar manner. The continuation of the same field for more than fifty years and the important results which subsequently followed from an examination of the soil so

long under definite cultivation may be cited as an example of Gilbert's methods. Under his care, samples of the grain and straw from each experimental plot in each year were preserved in the laboratory and also the samples of the soil and subsoils of each were repeatedly taken; large portions of each sample were also preserved. At his death the number of samples stored for future reference in the laboratory and in the adjoining building exceeded 50,000. The bulk of tabulated records prepared by the clerks at the laboratory was correspondingly large. He thus laid the foundation of solid work. The investigations made at Rothamsted on the non-assimilation of atmospheric nitrogen by farm crops, which were published in 1861, were accepted as conclusive evidence upon this much-disputed question. The work of Lawes and Gilbert on manures, nitrification, the nitrogen supply of crops, and the increase and decrease of nitrogen in the soil when different crops were produced, has had a most important bearing upon the maintaining of the fertility of soils. The general plan of the field experiments at Rothamsted has been to grow some one of the most important crops of rotation separately for many years in succession on the same land without manure, with barnyard manure, and with a great variety of chemical manures. The same kind of manure was applied each year upon the same plot. Experiments with different manures on mixed herbage, on prominent grasslands, on the effects of fallow, and on the actual course of rotation without manure, and with different manures, have likewise been made.

The association between Lawes and Gilbert in their scientific work is one of the most pleasant recorded of men of science. Dr. Gilbert as a rule spent an hour at Rothamsted every day that Lawes was at home. The plans for the new experiments, the results obtained from day to day, and the drafts of the reports in preparation were thus all discussed by them together. They worked as harmoniously as two brothers, and this lasted for a period of fifty-seven years, or until the death of Dr. Lawes in 1900. Dr. Gilbert died one year later.

Our modern science of bacteriology was, in a large measure, the outgrowth of the early work in agricultural chemistry. As the men studied and worked they found that chemical explanations were not sufficient, and from the earliest times until the present the chemist has made use of all the other sciences, indiscriminately, to aid in the solution of his problems.

The pioneers in agricultural chemistry first confined their attention to the chemical phases of plant growth. Ingen-Housz and de Saussure showed that atmospheric carbon dioxide was the source of carbon in plant compounds. Later Lommel and

Pfeffer emphasized the importance of light in the synthesis of organic compounds in the plant, and Sachs, Loew, Baeyer, and others pointed out the important function of the green pigment, chlorophyll, in this synthetic process. It has remained for Willstätter and his students to shed further light on the chemical composition of chlorophyll.

THE BEGINNINGS OF PHYSIOLOGICAL CHEMISTRY

Physiological chemistry or animal chemistry is an outgrowth of animal physiology. Experimental physiology was recognized as an independent division of science as early as 1800. To be sure, William Harvey had announced his discoveries regarding blood circulation as early as 1628. Similar progress had been made in the study of respiration. Robert Boyle had studied the effect of low and high air pressures on animals as early as 1659. Other important information regarding respiration was added by Robert Hooke (1667), John Mayow (1668), Joseph Priestley (1774–1777), and Antoine-Laurent Lavoisier (1777). However, it was not until the latter part of the nineteenth century that experimental physiology really became recognized as a separate division of science.

The same trends were characteristic of the fields of research on digestion and metabolism. Sanctorius of Padua (1614) had called attention to “insensible perspiration” and gain and loss of body weight as affected by food intake. Regnier de Graaf (1664) had described the properties of pancreatic juice, and Lazzaro Spallanzani (1782) had described the effect of saliva and gastric juice on foods. No important chemical techniques were advocated, although Dr. William Prout (1785–1850) identified hydrochloric acid in gastric juice. Prout is referred to by many writers as the first English physiological chemist. Dr. William Beaumont, American physician, published his classical work on gastric digestion in 1833. During the same period (1600–1850) similar progress had been made in many other phases of experimental physiology.

However, it was not until the period 1850 to 1880 that experimental physiology and physiological chemistry really achieved some degree of recognition. Even as late as 1880 to 1900 there

seemed to be no great appreciation of the value of the application of chemistry to physiology. Dr. Russell H. Chittenden, in his *Development of Physiological Chemistry in the United States*, credits Sir Michael Foster with the establishment of the first practical instruction of physiology in England in 1874.

Henry P. Bowditch, who had studied under Carl Ludwig in Leipzig, returned to the United States in 1871 and accepted the chair of physiology at Harvard University. Since at that time laboratory work was not considered essential, he had few facilities for experimental work. Nevertheless, with a few pieces of apparatus he had brought from Germany he set up a small laboratory in the attic of the medical building. This was the *first physiological laboratory for students in the United States*.

It was for the reasons just described that physiological chemistry was slow in developing. It was a borderline science, claimed by chemistry on the one hand and by physiology on the other. Even today departments of physiological chemistry may be found which are administered by other administrative divisions, such as medicine, biology, and physiology.

Even in Germany, where physiology and physiological chemistry had made great progress, editors and administrators were not quite sure where physiological chemistry belonged. It is interesting to record that the German edition of Berzelius' *Lehrbuch der Chemie*, translated by Friedrich Wöhler in 1840, had as the title of the ninth volume, *Thier-Chemie* (*Animal Chemistry*) and dealt with the chemistry of body tissues and body processes.

In 1826 and 1844 two books were published entitled *Physiological Chemistry*. The first of these was by Hünefeld, and the other was by Mülder and Marchand. In 1846 Justus von Liebig published his work, *Die Thier-Chemie oder die Organische Chemie in ihrer Anwendung auf Physiologie und Pathologie*. This book played a very important role in stressing the importance of chemistry in its application to animal physiology. However, medical men were slow in recognizing the importance of chemistry in medical research although physiology and physiological methods of research were generally accepted.

The period 1870 to 1880 was marked by brilliant researches of French and German physiologists. Claude Bernard of Paris

and Carl Ludwig of Leipzig were probably the most outstanding men of the period. Bernard's researches showed great versatility, and his contributions included the discovery of liver glycogen and its relation to blood sugar in health and disease, the digestive properties of pancreatic juice, and studies in muscle and nerve physiology. He was even more outstanding as a teacher and a creator of research techniques.

Carl Ludwig was appointed Professor of Physiology at Leipzig in 1865. Students from all over the world flocked to his laboratory. He worked closely with E. Drechsel, physiological chemist at the same institution. During this period Germany was a mecca for foreign students seeking advanced training in the physiological and chemical sciences.

"Household names" of this productive period were Du Bois-Reymond (Berlin), Baumann (Berlin), Heidenhain and Röhmann (Breslau), Pflüger (Bonn), Pettenkofer and Voit (Munich), Hoppe-Seyler (Strassburg), Hüfner (Tübingen), and Kühne (Heidelberg). From these laboratories came a host of young workers who were to carry on the future development of physiology and physiological chemistry.

Prior to 1877 there were no journals devoted to researches in physiological chemistry. In 1877 Hoppe-Seyler established the German journal *Zeitschrift für Physiologische Chemie*, which was devoted solely to papers on physiological chemistry. This journal was the forerunner of many others, and it played an important part in establishing physiological chemistry as a separate branch of chemical (and medical) research.

Up to this point we have emphasized the factors affecting the development of physiological chemistry in Europe. It was natural, therefore, that early American physiological chemists should look to Europe for inspiration and ideas.

Professor Russell H. Chittenden was trained at Yale University where the first well-organized course in physiological chemistry for medical students had been established in 1874. In 1878 Professor Chittenden went to Heidelberg where he studied with Professor Kühne, who had been trained in the laboratories of Virchow (Berlin), Ludwig (Leipzig), and Bernard (Paris). As a result of Chittenden's training it was but natural that Yale

University should become the center of physiological chemistry in America.

Dr. Chittenden was an enthusiastic and inspiring teacher and research worker. It was not long before students were flocking

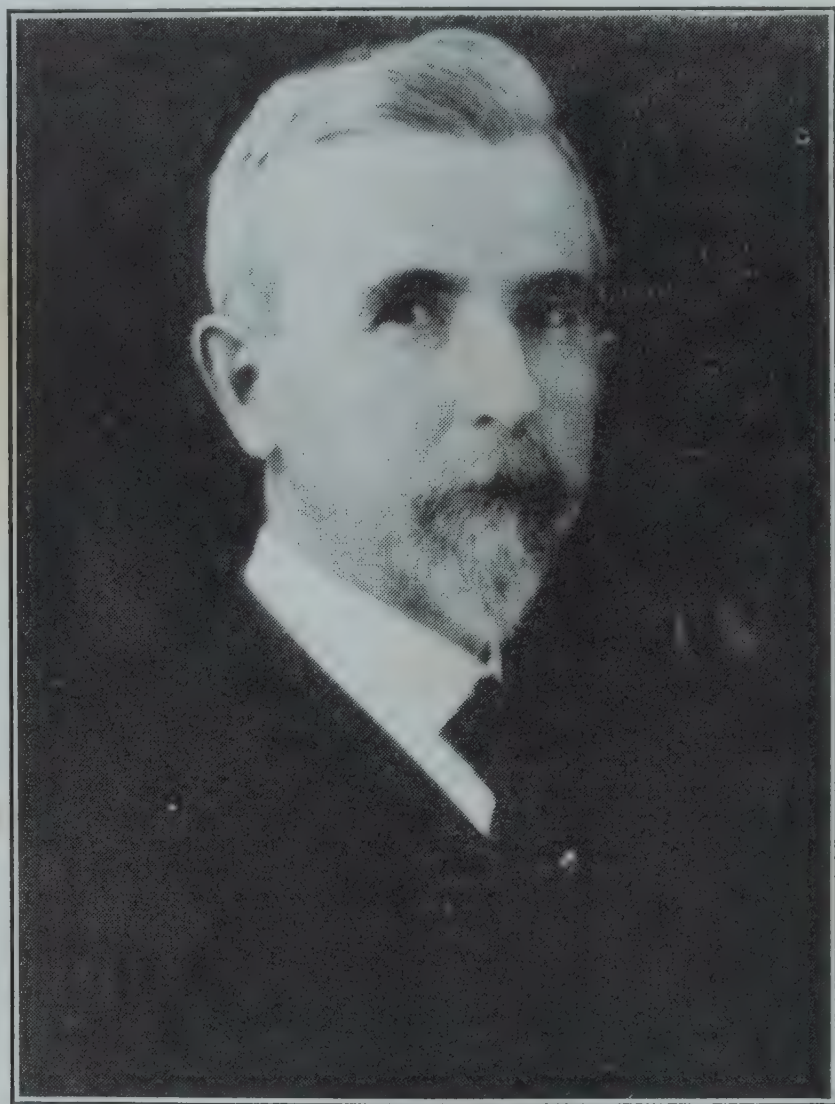


FIG. 3. Dr. Russell H. Chittenden (1856–1943), dean of American physiological chemists, whose laboratory at Yale University became the center of physiological chemistry in America. He is affectionately known as the Father of Physiological Chemistry in the United States.

to his laboratory from this and other countries. As a result most of the biochemical laboratories in this country are staffed with men and women who can trace their biochemical lineage to Dr. Chittenden. This was brought out quite dramatically at a dinner given in Dr. Chittenden's honor at Washington in March 1936. There were about 500 scientists at the dinner. At the termination of Dr. Chittenden's address, Dr. John R. Murlin asked all "biochemical sons, daughters, grandsons and

granddaughters" of Dr. Chittenden to rise and stand in their places. A rapid count showed that about 400 of the scientists present had been trained by Dr. Chittenden or by his students.

At first, research papers on biological and physiological chemistry were published wherever editors would accept them. The *American Chemical Journal*, which was the forerunner of the *Journal of the American Chemical Society*, was established by Ira Remsen in 1879. This journal accepted articles in all fields, including agricultural and physiological chemistry.

Gradually, specialized scientific groups organized their own journals in specialized fields. The literature grew rapidly, and abstract journals were created to assist the busy teacher and research worker in keeping abreast of the world literature. As a result American biochemistry grew and flourished until today this country stands second to none in this important field of science.

The American Chemical Society, organized in 1876, is now the largest society of its kind in the world, with a membership of more than 60,000 chemists. In August 1941, according to a survey conducted by the American Institute of Chemists, there were 1600 chemists and 150 chemical engineers employed by the Federal Government. Many others have been added as the result of the establishment of new research projects growing out of the second World War. Many, if not most, of the chemists employed by the Federal Government are assigned to problems which are primarily biochemical in nature, so far as final objectives are concerned.

BEGINNING OF AGRICULTURAL SCIENCE IN AMERICA

Probably the first American publication (1678) which called attention to the chemical phases of agriculture was a paper by John Winthrop, Jr., the first governor of Connecticut. In this paper, "The Description, Culture and Use of Maize," he called attention to the sugar content and described the syrup obtained therefrom. In 1688 the Reverend John Clayton emphasized certain chemical properties of tobacco. In these early pre-revolutionary days there was a growing interest in chemistry

as it related to agriculture, but no chemical work is on record.

One of the earliest chemists who exerted a real influence on American agriculture was Dr. Samuel L. Mitchill, who held the chair of chemistry and agriculture at Columbia College from 1792 to 1801. Professor Mitchill helped to found the New York Society for the Promotion of Agriculture, Manufactures and the Useful Arts, and through his efforts considerable interest was developed in the use of gypsum as a fertilizer.

In 1806, Dr. Thomas Ewell, of Virginia, published an important treatise in which he stated that "Agriculture is most intimately connected with chemistry. The power of seed to attract and unite to parts of the soil, so as to vegetate or increase in bulk, is purely chemical. Chemical knowledge will teach the gardener and farmer what particular soil is best adapted for particular seed; it will teach the way of forming soils for foreign plants; of making manures to the greatest advantage; of preserving grains, roots, etc.; of destroying the insects; and of correcting the disorders injuring the valuable shrubs."

Sir Humphry Davy's work, mentioned in a previous paragraph, was published in 1813 and went through several American editions. This was the standard book in agricultural chemistry until the appearance of Liebig's well-known work in 1840. Both men exerted a marked influence on American agriculture of that period.

In 1810 Dr. Gerard Troost, a Dutch physician, emigrated to this country. He not only made what appear to be the earliest studies of the composition of Pennsylvania soils, but he made valuable contributions to agriculture, mineralogy, and geology in Tennessee, where he spent the remainder of his life.

The first chemical bulletin pertaining to agriculture, published by the government, was written by Benjamin Silliman, Sr., Charles Upham Shepard, and collaborators. This work, which was devoted to the chemistry of sugar cane, was published in 1833. In 1834 Mr. Shepard was made professor of chemistry at the South Carolina Medical College. While residing at Charleston, Mr. Shepard discovered the calcium phosphate deposits which were destined later to yield enormous revenues in the manufacture of fertilizers.

Edmund Ruffin, in 1821, published an *Essay on Calcareous Manures* which, greatly enlarged, was issued in book form in

1852. This work has been described by Dr. C. A. Browne as a "classic" of its time "which can still be consulted with profit."

In 1842 Dr. Samuel Luther Dana published his *Muck Manual*, which exerted a tremendous influence on New England agriculture for the next twenty years. The period 1840 to 1850 was marked by a considerable increase in scientific and industrial activities. Chemists were busy studying the chemistry of soils, crops, foods, and fertilizers.

A brilliant young agricultural chemist by the name of John Pitkin Norton was professor of agricultural chemistry at Yale from 1847 to 1852. Although Professor Norton died at the early age of thirty, he had found time to be an inspiring teacher, a brilliant research worker, and a copious writer. As a result of Norton's influence Samuel W. Johnson and William H. Brewer, both of whom became celebrated agricultural chemists, became interested in agricultural chemistry. Professor Johnson, who later succeeded Professor Norton at Yale, is probably best known for his books entitled *How Plants Feed* and *How Crops Grow*.

No discussion of American agricultural chemists would be complete without a word or two about Dr. Evan Pugh. Like Professor Norton, Dr. Pugh died early in life. In spite of this he crowded into his busy young life accomplishments that would have done credit to a man of considerably greater age. Dr. Pugh obtained his graduate chemical training in Europe, and after he had obtained his doctorate he spent two years at the Rothamsted Experiment Station where, with Lawes and Gilbert, he published his well-known treatise entitled *Sources of the Nitrogen of Vegetation*. In 1859 Dr. Pugh returned to the United States in order to accept the presidency of the Pennsylvania State College and headship of agricultural chemistry at State College, Pennsylvania, where he died a few years later at the age of thirty-six years. Although Dr. Pugh's researches were not numerous, they were characterized by an unusually high quality of skill and accuracy. His administrative record at the Pennsylvania Agricultural College was no less brilliant, and the organization of the college curriculum, the inauguration of research, and the high standards set by Dr. Pugh at that early date will always stand as monuments to his memory.

One of the most important contributions made by the Pennsylvania State College to American agriculture was the establish-

ment, in 1881, of the Soil Fertility Plot Experiments which have been continued to the present day. The fiftieth anniversary of the establishment of these experiment plots was celebrated at the Pennsylvania State College in July 1931, and was attended by prominent soil scientists from this and other countries. During the anniversary celebration the plots were dedicated to Dr. Whitman Howard Jordan, who laid them out in 1881. They are now known as the Jordan Soil Fertility Plots. It is to be regretted that Dr. Jordan died but a few weeks prior to the time that he was to be the guest of honor at the fiftieth anniversary celebration.

Our brief description of prominent agricultural chemists will not be complete without the mention of Professor John W. Draper, the first president of the American Chemical Society. In 1844 Professor Draper published his *Treatise on the Forces which Produce the Organization of Plants*. This work attracted considerable attention, particularly with reference to the photosynthetic processes in plants.

Professor Samuel W. Johnson of Yale undoubtedly exerted a greater influence than any other agricultural chemist of his time. Though Professor Johnson achieved a reputation as a research worker, he is best known for his ability as an organizer, administrator, and writer. He is considered one of the most capable critics of agricultural chemical literature of all time. Professor Johnson is known as the founder of chemical regulatory work in America, owing to his inauguration of fertilizer control work in the State of Connecticut.

If space permitted, we should like to discuss invaluable contributions to American agriculture made by outstanding contemporary agricultural chemists. We should like to tell the reader of the work of Professor E. W. Hilgard, Dr. Cyril Hopkins, and others in the field of soil chemistry; of Dr. S. M. Babcock in dairy chemistry and animal nutrition; of Dr. C. A. Goessmann and Dr. Whitman H. Jordan in animal nutrition, fertilizer studies, and research administration; of Dr. L. L. Van Slyke in dairy chemistry; of Dr. Henry Prentiss Armsby in animal nutrition; of Dr. William Frear in soils chemistry and pure food work; and of many others of equal prominence whose contributions will be described in succeeding chapters.

That the chemist has played an unusually important role in American agricultural development is brought out best by Dr. Harvey W. Wiley, who stated in an address before the American Chemical Society in 1901, "Of the forty-nine directors of the stations at the present time, twenty were professional chemists at the time of their appointment. The selection of so many professional chemists was no mere chance, but evidently had some relation to the dominant position which the science of chemistry holds to the promotion of agricultural research. The list of the directors of agricultural experiment stations of Germany shows the same condition of affairs."

In the United States no greater encouragement has been offered to agricultural science than the grants made at different times by the Federal Government. The Morrill Act in 1862 established our agricultural colleges; the Hatch Act in 1887 established our agricultural experiment stations; and the Adams Act in 1906 provided means for special investigations which have meant much for the promotion of agricultural research. Passage of the Purnell Act (1925) and the Bankhead-Jones Act (1935) which provide additional funds for the prosecution of scientific research in the various state agricultural experiment stations, have served to stimulate many phases of scientific activity as well as the creation of many new research projects. In 1946 Congress passed the Research and Marketing Act which provides additional funds for investigations of "basic laws and principles relating to agriculture and to improve and facilitate the marketing and distribution of agricultural products."

Another Federal agency of inestimable value to American agriculture is the Department of Agriculture at Washington, D. C. With its many bureaus, one of which is the Bureau of Agricultural Chemistry and Engineering, and its excellent scientific personnel, not only has it played a most important role in scientific agricultural development, but its researches have helped to better living conditions in every walk of life in every state of the union.

The establishment in 1938 of Regional Research Laboratories for the study of special problems relating to agriculture promises even greater governmental assistance in the solution of difficult problems peculiar to the various regions of the country.

2 · Chemistry of Living Matter

Biochemistry has been defined as the “chemistry of living things.” As a result, much of the chemistry with which we are to deal in this book will have to do with the chemistry of life processes.

In general we can classify all things with which we are familiar into two classes, the *living* and the *non-living* or lifeless. What properties do all living things possess that differentiate them from non-living things? One property comes to mind immediately, namely, *the power of movement*. To be sure, plants cannot move from place to place, like animals, but certain plant organs are capable of movement to a limited extent.

A second property, *growth*, is characterized by synthetic development from within which is different from the type of growth we see in inanimate crystals which grow by additions from the outside. A third property of living matter is the *power to respire*. During respiration living tissues undergo characteristic oxidative changes, consume oxygen, give off carbon dioxide, and liberate energy. In addition all living organisms respond to environmental stimuli. Consequently the biologist tells us that living organisms possess the *property of irritability*. This is a fourth property possessed by living things. Finally, and possibly most important, is the power of living organisms to reproduce. If it were not for the *power of reproduction*, all living matter would cease to exist.

All the above-mentioned properties of living matter depend on controlled chemical reactions. These controlled reactions, in turn, depend on the unique ability of living cells *to elaborate essential controlling agents, called enzymes*.

The science of biology teaches us that the cell is the biological unit of life. Chemistry and physiology have shown that these tiny units may be likened to factories, each receiving its raw materials from which it fabricates not only its own building materials but, in addition, furnishes essential materials for other cells elsewhere in the living organism.

The cell. Animal cells are covered by a protoplasmic membrane, whereas plant cells usually possess two membranes, one consisting of modified protoplasm similar to that of the cell itself, and a second membrane or heavy wall consisting of cellulose.

The living plant cell is, in reality, a protoplast, i.e., a droplet of organized protoplasm enclosed in a cellulose compartment. When such a cell is plasmolyzed by immersion in a salt solution of higher concentration than that of the cell itself, the cell loses its characteristic turgidity and the protoplasmic membrane draws away from the cellulose covering.

The typical cell contains a spherical or semispherical body called a *nucleus*. If the nucleus is removed, the cell loses its power of reproduction. Certain cells have several nuclei, and a few are non-nucleated. Examples of non-nucleated cells are the red blood cells in man.

Although the cytologist and histologist are interested in the details of cell structure, the biochemist is interested primarily in the chemical composition of the cell and in the chemical changes which occur during cell metabolism.

Although cells of plants and animals differ in many respects, we find that both types of cells contain, as their principal structural substance, a liquid or semiliquid form of matter known as *protoplasm*. Careful chemical studies of plant and animal protoplasm indicate that both types are essentially similar in their chemical and physical characteristics.

Protoplasm. Protoplasm is a translucent, grayish, shiny substance similar to a thin jelly in consistency. To use another example, protoplasm has properties similar to those of fresh egg white. Under the microscope it appears to consist of a thick matrix containing globules and granules. Chemically, protoplasm seems to be a colloidal system consisting of water (about 75 per cent) and solid matter (about 25 per cent). Protein

seems to be the major solid constituent. Other chemical constituents of protoplasm include sugars, lipids, amino acids, and inorganic salts.

At first glance it would appear that protoplasm is a rather simple system. However, it is a very complex system when considered from the dynamic standpoint. Thousands of diverse activities may be ascribed to protoplasm. Since protoplasm is a colloidal system (emulsion), we will learn in a later chapter (Chapter 3) that many of the characteristic properties of protoplasm can be explained only in terms of physics and physical chemistry. This colloidal system contains proteins, lipids, and other chemical substances which possess the unique property of attracting and holding large amounts of water within the protoplasmic structure. The presence of water gives protoplasm its characteristic jellylike properties causing it, at times, to behave as a semiliquid and, at other times, as a semisolid. Consequently the behavior of cell membranes, the permeability of cells, and the metabolic changes within the cells must depend, in great measure, on the condition of the protoplasm at any given moment.

The turgidity of cells depends upon interrelationships existing between water and other cell constituents. Consequently the uptake (imbibition) of water by cells, the viscosity of protoplasm, and the elasticity of the cell membrane require very delicate balance and control, details of which still baffle scientific workers.

Naturally a host of questions suggest themselves regarding this protoplasm: what is its function in the cell and what are the chemical activities of the cell itself; how does it receive its raw materials; what changes are produced and how are these chemical changes stimulated and controlled? Many of these questions can be answered by means of chemical research, and it is through the study of problems of this type that we are able to obtain a mental picture of the various phases of growth and development in plants and animals. However, before we can go far in the study of these questions we must ascertain, so far as possible, the chemical composition of the cell and the nature

and structure of the constituents, as well as their chemical and physical properties.

IMPORTANCE OF WATER

*Alles ist aus dem Wasser entsprungen,
Alles wird durch das Wasser erhalten.*

Goethe (*Faust*)

It is impossible to overstress the importance of water in living processes. Water is the solvent and dispersion medium for all protoplasmic constituents. Water not only acts as a transportation medium for cell nutrients throughout the living organism, but it also serves as the medium in which reacting substances undergo fundamental changes. It is only necessary to cite the ascent of sap in trees and the circulation of blood in animals to emphasize the importance of water for the maintenance of life. Absorption, secretion, and excretion would not be possible without water.

From a biological standpoint it is important that water exhibits high surface tension, that it forms hydrates with many compounds, that it possesses high specific heat, and that it is a good conveyor of heat. Water is necessary for the hydrolytic splitting of carbohydrates, fats, and proteins. The plant requires from 200 to 400 pounds of water to produce 1 pound of dry matter. In spite of this, the plant maintains a very accurate balance between water and other normal cell constituents.

Water exists in living tissue as *free water* and as *bound water*, or water of hydration. Both forms of water are of great biological importance. Workers at the University of Minnesota have shown that winter hardiness in wheat is a function of the proportion of free water to bound water existing in growing tissues. Free water freezes, whereas bound water does not form ice crystals in the tissues. Water cannot pass freely in and out of living cells because its passage is controlled by osmosis, hydration of colloids, and other factors.

In certain diseases of man the permeability of cell membranes is altered, and normal water balance is upset. An example is a kidney disease, known as *nephritis*. In this disease, tissues swell

to abnormal size owing to excessive water uptake. The condition is commonly known as "dropsy." Epilepsy is associated with excessive water supply and alkalinity; diabetes is associated with dehydration and acidity. Excessive losses of water by sweating deplete the body tissues of essential mineral salts to the point where salt depletion may cause prostration.

As tissues age they lose their normal water-holding ability. As a result tissues of young organisms are richer in water than those of aged organisms. Mammals, such as dogs and man, can survive for a month or more without food if water is available. However, death will occur in but a few days if the body is deprived of water.

When deuterium (heavy hydrogen) unites with oxygen, *heavy water* is formed. Oxygen also exists in isotopic forms with atomic weights of 16, 17, and 18, respectively. Theoretically the two isotopic forms of hydrogen and the three isotopic forms of oxygen are capable of uniting to form nine different kinds of water. Heavy water and isotopic forms of hydrogen and oxygen serve as new tools for the biologist and biological chemist in their studies of cell metabolism.

Inorganic salts. Mineral elements occur in living tissues as inorganic salts, as salts of organic acids, and in combination with organic compounds. Although sulfates, chlorides, phosphates, and carbonates of sodium, potassium, calcium, and magnesium are usually considered most important, salts of trace elements are often present in significant amounts. Examples of such micronutrient elements are boron, copper, cobalt, manganese, iodine, and zinc.

Phosphorus and sulfur are unique in that they form vital combinations with organic compounds. For example, certain coenzyme systems, essential for normal metabolism, contain phosphates as part of the coenzyme molecule. Certain essential amino acids contain sulfur in highly reactive form. These and other inorganic elements will be discussed in subsequent chapters.

Proteins, lipids, and carbohydrates contain carbon, combined in various combinations with other elements, of which hydrogen, oxygen, nitrogen, and sulfur are most important. These compounds play a major role in normal plant and animal growth. In subsequent chapters we shall consider the chemical structure

and properties of carbohydrates, lipids, and proteins in considerable detail.

The following chapter will introduce the reader to those phases of physics and physical chemistry which are necessary for a better understanding of the physical and chemical changes that occur in living tissues.

3 · Physical State of Matter

In the preceding chapter we have considered the chemistry of living matter, emphasizing the *chemical properties* of the various types of compounds and stressing the *chemical composition* of plant and animal materials. In this chapter we shall consider the more important *physical* and *physicochemical properties* of protoplasm and its various constituents.

When we begin to study the various body fluids and tissues, we are impressed with the large number of variables that regulate our physiological processes. For example, it is impossible to go far in the study of protoplasmic activity without realizing that the physical sciences are of great importance. We are forced to consider, therefore, such topics as dissociation, electrical charges, conductivity, osmosis, surface tension, and colloids, as well as a whole host of other interesting phenomena, before we can even begin to approach an understanding of protoplasm.

SOME PROPERTIES OF SOLUTIONS

Dissociation. Aqueous solutions may be divided into two classes, namely, those that conduct electricity and those that do not. Aqueous solutions of inorganic acids, bases, and salts are conductors of electricity and are, therefore, called *electrolytes*. In general, organic compounds are poor conductors of electricity and are called *non-electrolytes*.

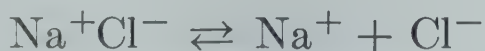
Whereas non-electrolytes give normal values for osmotic pressure, boiling-point rise, and freezing-point lowering, it has been found that electrolytes give abnormal values. In 1887 Arrhenius

proposed a theory of electrolytic dissociation which serves to explain these phenomena. He postulated that electrolytes dissolved in water are dissociated into charged ions and that the electric current is transmitted through these charged ions. *Dissociation, therefore, is the separation of an electrolyte into its constituent ions* and may be represented as follows:



This equation indicates that an equilibrium is established between NaCl and its respective ions. Arrhenius suggested that at infinite dilution dissociation is complete, but that as the concentration of the solution increases a part of the original solute remains in solution in an undissociated state. Therefore in a dilute solution the equilibrium is shifted toward the right, whereas in concentrated solutions the equilibrium tends to shift toward the left.

In recent years the theory of Arrhenius has been altered somewhat by the research work of the physicist and the physical chemist. X-ray studies of a crystal of sodium chloride, for instance, indicate the presence of sodium and chloride ions spaced at equal distances from each other throughout the crystal structure. Accordingly it is suggested that electrolytes such as sodium chloride are completely dissociated in the solid state as well as in solution. Theoretically, therefore, the above equation should be modified as follows:



This equation may be interpreted to mean that, when an electrolyte such as NaCl is dissolved in water, *pre-existing ions* are dissociated rather than *pre-existing molecules*.

Osmosis and osmotic pressure. Another property of solutions which plays a most important part in all living tissues is that of osmosis and the creation of osmotic pressure. In general chemistry it was pointed out that, when two gases are brought into contact, they diffuse, one into the other, by reason of molecular motion, tending eventually to come to equilibrium in a uniform mixture. During the process of diffusion the difference in partial pressures of the two gases produces a force which is measurable.

A similar phenomenon may be observed when two aqueous mixtures of different concentration are brought together. Owing to the greater friction between the molecules of the solute and those of the solvent, the diffusion will progress less rapidly, but eventually the solutions tend to come to equilibrium and the mixture is uniform throughout.

If a semipermeable membrane (one permeable to the solvent but not to the solute) separates water from a water solution of sucrose, it will be found that water will pass through the membrane and dilute the sucrose solution. This flow is called *osmosis*, which may be defined as the passage of a solvent through a semipermeable membrane from a dilute solution to a more concentrated one. When osmosis occurs, a pressure develops as the result of the dilution of the solution by the solvent. This pressure, known as *osmotic pressure*, is proportional to the mole fraction of solute molecules in the original solution.

If sufficient external pressure is applied to the solution, the passage of solvent will be reversed (i.e., from solution to solvent). Accordingly osmotic pressure may be defined as the least amount of pressure which must be applied to a solution to prevent osmotic flow of the solvent into the solution. The fact that external pressure can be applied easily to a solution (by means of a column of mercury, a piston device, or a large column of the solution itself) permits the investigator to measure the osmotic pressure of a solution very accurately.

Theoretically a semipermeable membrane is permeable to the solvent but not to the solute. Actually, however, most membranes are not strictly semipermeable; that is, some simple solutes are allowed to pass through. This fact makes it possible to separate simple solutes from more complex solutes by allowing the simple ones to pass through the membrane. This separation process, known as *dialysis*, is of practical importance in the purification of many compounds.

Surface tension. Liquids possess another property of biological importance, namely, surface tension. The surface of a liquid possesses certain properties which account for this phenomenon. Molecules at the surface of a liquid are pulled inward by similar molecules located in the liquid. Thus the liquid tends to adjust itself to give a minimal surface area. Many natural phenomena such as the spherical shape of raindrops and the movement of

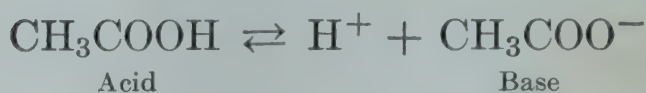
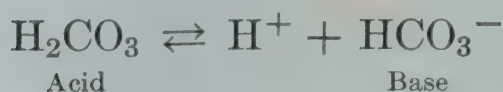
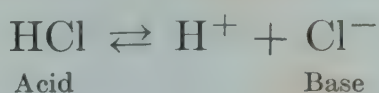
water in the soil or in blotting paper are due primarily to the surface tension of the liquid in question. Thus we may imagine that the surface layer of a liquid acts as an elastic film which, like rubber, tends to contract and become smaller. This force which acts to decrease the surface may exist at the interface between gases and liquids, between gases and solids, between liquids and solids, or between two immiscible liquids. When liquid-vapor interfaces are considered, the term *surface tension* is used, whereas the term *interfacial tension* refers to those types represented by liquid-liquid or solid-liquid interfaces. Whereas some liquids, like water, wet the walls of a capillary tube, others such as mercury, do not. When a liquid wets the capillary, the body of the liquid is pulled up into the tube. The height to which the liquid will rise in the capillary is a function of the surface tension of the liquid. Therefore an accurate method for the determination of surface tension of a liquid consists of measuring the height to which the liquid rises in a capillary tube of known diameter.

Free acids and bases, particularly organic acids and bases, usually decrease the surface tension of water. Electrolytes, as a rule, have little effect. Non-electrolytes such as aldehydes, monohydroxy alcohols, ketones, and esters of the organic acids tend to lower the surface tension of water. Bile salts, lecithin, starch, and glycogen also possess a lowering effect. As a result we might expect that physiological solutions, such as milk, urine, blood serum, and bile, would have a lower surface tension than that possessed by pure water, and such has been found to be true.

Those substances that tend to lower surface tension become concentrated in the surface layers (positive adsorption); those that tend to raise surface tension are found in greater concentration in the body of the solution (negative adsorption). When egg albumin lowers the surface tension of water, it also increases its viscosity. Concentration of the albumin in the surface layer leads to the development of a film. If air is passed through the mixture the formation of a froth or foam is brought about through the formation of viscous films. Foaming or frothing may be said to be encouraged by increased viscosity, and any substance that will lower viscosity will discourage the formation of foam.

In other words, any substance that lowers viscosity and increases surface tension will discourage foam formation.

Acids and bases. Arrhenius defined an acid as a substance that yields hydrogen ions in solution, and a base as a substance that yields hydroxyl ions in solution. According to the more modern theory of Brønsted, however, an acid is a proton donor whereas a base is a proton acceptor. In equation form, Brønsted's theory may be stated as follows:

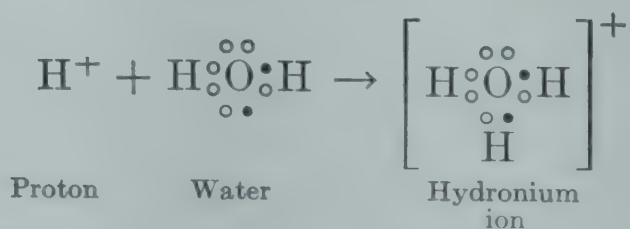


Whereas the Brønsted theory does not materially change the definition of an acid, it does change our concept of a base. The Arrhenius definition of a base was formulated with the assumption that water is the solvent, whereas the Brønsted theory recognizes that there are many solvents other than water. In biochemistry, however, water is the most important solvent in body tissues and, accordingly, we can utilize the simpler though older theory and definition of acids and bases.

Dissociation of water. As has been stated, water is of prime importance as a solvent in biological materials. According to the theory of Arrhenius water dissociates as follows:

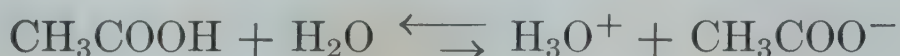
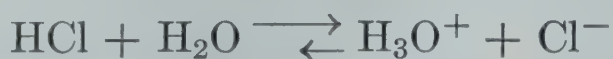


We learned in general chemistry that the hydrogen ion is actually a single proton. Recent evidence indicates that free protons cannot exist in a solvent such as water. It has been found that a water molecule shares one of its lone pairs of electrons with the proton forming a dative bond as shown in the following equation:



According to this equation a new ion, the *hydronium ion*, is formed. Actually the hydronium ion (H_3O^+) is a hydrated proton found in aqueous solutions of all acids, and is responsible for the typical acid properties of such solutions.

In the light of this evidence aqueous solutions of acids such as HCl or CH_3COOH ionize as follows:



It would be wise for the student to familiarize himself with the theory of the hydronium ion even though in the general literature of chemistry the hydronium ion is still being called the hydrogen ion.

The fact that pure water exhibits a slight electrical conductivity indicates that ions must be present. The formation of these ions can be shown by the following equation:



By applying the law of mass action we have:

$$K = \frac{[\text{H}_3\text{O}^+][\text{OH}^-]}{[\text{H}_2\text{O}]^2}$$

and

$$K[\text{H}_2\text{O}]^2 = [\text{H}_3\text{O}^+][\text{OH}^-]$$

Since water is very weakly dissociated, the concentration of undissociated water, for all practical purposes, remains constant. Therefore for $K[\text{H}_2\text{O}]^2$ we can substitute a new constant, K_w , which represents the dissociation constant of water. In terms of this new constant we can write the equation:

$$K_w = [\text{H}_3\text{O}^+][\text{OH}^-]$$

Since the two ions, H_3O^+ and OH^- , are formed in equal quantities, it follows that their concentrations are equal. By measuring the dissociation constant for water we find that $K_w = 10^{-14}$. Since the concentrations of the ions are equal, it is obvious that $[\text{H}_3\text{O}^+] = 10^{-7}$ and $[\text{OH}^-] = 10^{-7}$, the concentrations being expressed in gram ions per liter.

Solutions in which the hydronium ion and the hydroxyl ion concentrations equal 10^{-7} are said to be *neutral*. A solution is

said to be *acidic* if the hydronium ion concentration exceeds the hydroxyl ion concentration, whereas a solution is said to be *basic* if the hydroxyl ion concentration exceeds the hydronium ion concentration.

Hydronium ion concentration and pH. Hydronium ion concentration may be expressed in terms of gram ions per liter. When dealing with biological materials, however, this involves the use of extremely small decimal fractions. Owing to the inconvenience of writing such decimals and more particularly because of the possible attendant errors, the system of *pH* was devised. *pH* may be defined as the logarithm of the reciprocal of the hydronium ion concentration:

$$pH = \log \frac{1}{[H_3O^+]}$$

It follows then that any solution whose hydronium ion concentration is 10^{-7} has a *pH* value of 7, since

$$pH = \log \frac{1}{10^{-7}} = \log 10^7 = 7$$

A *pH* of 7 indicates a neutral solution since a neutral solution by definition is one whose hydronium ion concentration is 10^{-7} .

If the hydronium ion concentration of a solution is 10^{-6} , then

$$pH = \log \frac{1}{10^{-6}} = \log 10^6 = 6$$

Thus a solution the *pH* of which is 6 has ten times the hydronium ion concentration of a solution with a *pH* of 7. Likewise a solution with a *pH* value of 5 is ten times as acid as one with a *pH* of 6, and one hundred times as acid as a solution with a *pH* of 7.

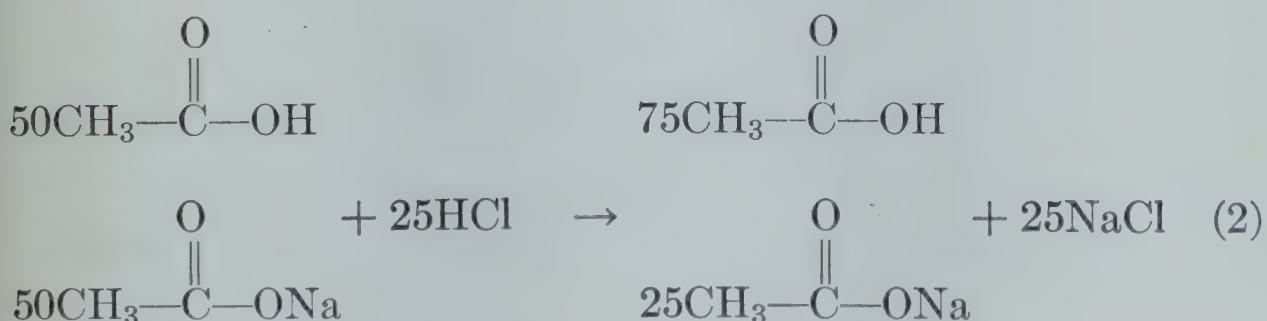
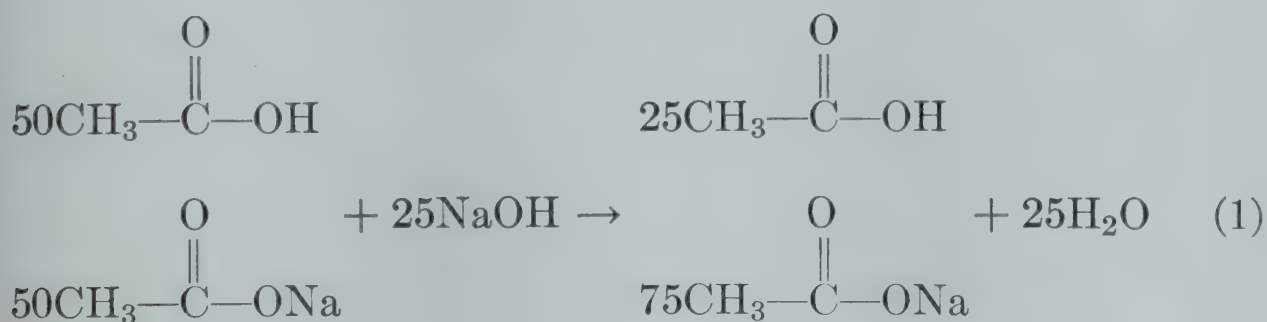
In a similar manner a solution with a hydronium ion concentration of 10^{-8} has a *pH* of 8 and is ten times as basic as a solution of *pH* 7. Thus all solutions with a *pH* less than 7 are acidic, and those with *pH* values greater than 7 are basic.

It must be remembered that, for all practical purposes, the terms "hydronium ion concentration" and "hydrogen ion concentration" are synonymous. Since "hydrogen ion concentration" is most frequently used in the present chemical literature, the authors will use this term throughout this book.

RELATIONSHIP BETWEEN HYDROGEN ION CONCENTRATION AND pH

Hydrogen Ion Concentration, moles/liter		Hydroxyl Ion Concentration, moles/liter	pH	Character
1	$1 = 10^0$	10^{-14}	0	<div> <div>↑</div> <div>Increasing acidity (decreasing basicity)</div> </div>
1/10	10^{-1}	10^{-13}	1	
1/100	10^{-2}	10^{-12}	2	
1/1,000	10^{-3}	10^{-11}	3	
1/10,000	10^{-4}	10^{-10}	4	
1/100,000	10^{-5}	10^{-9}	5	
1/1,000,000	10^{-6}	10^{-8}	6	
1/10,000,000	10^{-7}	10^{-7}	7	Neutral
1/100,000,000	10^{-8}	10^{-6}	8	<div> <div>↓</div> <div>Decreasing acidity (increasing basicity)</div> </div>
1/1,000,000,000	10^{-9}	10^{-5}	9	
1/10,000,000,000	10^{-10}	10^{-4}	10	
1/100,000,000,000	10^{-11}	10^{-3}	11	
1/1,000,000,000,000	10^{-12}	10^{-2}	12	
1/10,000,000,000,000	10^{-13}	10^{-1}	13	
1/100,000,000,000,000	10^{-14}	$1 = 10^0$	14	

Buffers are substances that prevent sudden or great changes in hydrogen ion concentration when strong acids or bases are added to a system. From a chemical standpoint buffers are composed of a mixture of a weak acid and its salt, or a weak base and its salt. An example of a buffer system and how it reacts toward strong acids and bases may be illustrated as follows:



In reaction 1 a strong base, NaOH, is added to the acetic acid-sodium acetate buffer system. The reaction produces more sodium acetate and water, neither of which affects the pH to a large extent. Since the hydroxyl ion from the NaOH is changed to water, the pH of the solution remains constant for all practical purposes, so long as some acetic acid remains.

In the second reaction the addition of HCl to the buffer results in the formation of more of the slightly ionized acetic acid. As in the first case the change in pH is negligible. It must be remembered, however, that there is a limit to the capacity of the buffer to take up HCl or NaOH. For example, if we were to add 50 NaOH in reaction 1, all the acetic acid would be neutralized and the next small amount of NaOH would result in an appreciable change in the pH of the system. The buffering capacity of a buffer system is at its maximum when the salt and the acid are in equal molecular proportions.

In living organisms, acids and alkalies are being liberated continuously. It is found that very slight changes in hydrogen ion concentration in the animal body are harmful. In other words, the animal organism must keep a very delicate balance between hydrogen ion and hydroxyl ion concentration. This is accomplished by a series of buffer substances. Examples of such buffers found in the body are the phosphates, the proteins, the carbohydrates, and the bicarbonates.

THE COLLOIDAL STATE

In 1861 Thomas Graham published a number of papers giving the results of his studies on the diffusion of certain substances through membranes. Some of these substances passed through membranes readily; others either diffused at a very slow rate or failed to do so entirely. Generally speaking, those that diffused readily were capable of crystallization, whereas those that did not were substances like glue, gelatin, and gums, always without form or amorphous in the solid state. To the first class he gave the name *crystalloids*, and to the latter the name *colloids*. The reader can understand this more readily when the following experiment is described. Common table salt and starch paste are placed in water in a parchment bag, and the bag is suspended

in distilled water. Chemical tests show that the salt passes through the membrane into the distilled water, whereas the colloidal starch remains in the parchment bag. We say that starch cannot pass through the membrane owing to its colloidal condition.

True solution, colloidal solution, and coarse suspension. Elementary inorganic chemistry teaches us that chemical substances are capable of existing in *true solution* in molecular and ionized form. The size of the particles in true solution has been placed arbitrarily at less than $1/1,000,000$ millimeter in diameter. $1/1,000,000$ millimeter is commonly designated as 1 millimicron ($m\mu$); a micron is $1/1000$ millimeter. Particles existing in *colloidal solution* are said to have diameters between 1 millimicron and 0.1 micron ($1/1,000,000$ millimeter to $1/10,000$ millimeter). *Suspensions* are made up of particles whose diameters are greater than 0.1 micron. Such particles will settle out on standing; hence the name, suspension.

Particles in true solution display molecular motion, whereas particles in colloidal solution show a peculiar type of motion known as *Brownian movement*. If the particles of a colloidal solution are observed by means of the ultramicroscope, the observer will notice that the particles do not move smoothly in the solution but seem to vibrate in a fixed position. These vibrations, known as Brownian movement, result from the bombardment of the colloidal particles by the ions and molecules of the solution. Suspensions exhibit slow Brownian movement as well as gradual gravitational movement.

When a beam of light passes through a true solution, the solution appears to be transparent. A beam of light causes most colloidal solutions to take on a hazy appearance, owing to a reflection of the light by the colloidal particles. This property of colloidal solutions is referred to as the *Tyndall phenomenon*. Suspensions are opaque and reflect light.

The table on p. 42 summarizes the properties of true solutions, colloidal solutions, and suspensions.

In a previous paragraph we learned that those substances which decrease surface tension tend to concentrate in the surface layers. Therefore, when this occurs, substances tend to concentrate in the surrounding film or adhere to the colloids, in the

PROPERTIES OF TRUE SOLUTIONS, COLLOIDAL SOLUTIONS, AND SUSPENSIONS

Property	True Solutions	Colloidal Solutions	Suspensions
1. Particle size	$<1\text{ m}\mu$	$1\text{ m}\mu\text{--}0.1\text{ }\mu$	$>0.1\text{ }\mu$
2. Visibility	Invisible	Ultramicroscopic	Microscopic
3. Movement	Molecular	Brownian	Brownian and gravitational
4. Osmotic pressure	High	Low	None
5. Filterability	Pass through membranes and filters	Pass through filters but not through membranes	Will not pass through filters or membranes
6. Effect of lateral illumination	Transparent	Tyndall cone	Opaque

manner described. When this situation exists, the substances are said to be *surface tension active* and are *adsorbed*. The process is known as *adsorption*.

Adsorption plays a most important role in biology and industry, for there is scarcely a phase of life that is not affected by the concentration of substances on colloid surfaces by the adsorption process. The decolorization of sugar solutions in industry by charcoal is a case in point, the enormous surface offered by the finely divided charcoal being capable of adsorbing large amounts of colored impurities. Adsorption is, therefore, a surface phenomenon which is probably physical and chemical in nature. To give the reader some idea of the enormous surfaces that finely divided colloidal particles may possess, let us take for example the red blood corpuscles, which are much larger than colloids and which possess much less surface area.

Assuming that there are 5,000,000 red blood corpuscles in 1 cubic millimeter of blood and assuming the average diameter of a single corpuscle to be 0.007 millimeter, it is calculated that the red corpuscles in 5000 cubic centimeters of blood (about this amount for a man weighing 150 pounds) would offer a surface area for adsorption purposes of 18,750 square feet or nearly one-half an acre. It is not difficult to understand, therefore, why certain colloidal materials are capable of adsorbing relatively large quantities of chemical substances. Colloids not only adsorb foreign materials but also mutually adsorb one another,

and for this reason it is almost impossible to purify chemical substances which have a tendency to exist in the colloidal state, on account of the adsorption of extraneous materials. For this reason, as we have pointed out elsewhere, it is very difficult to obtain proteins in sufficiently pure form to study them with the same accuracy that is possible in the case of easily crystallizable substances.

It has been found that colloids have the power, on account of their surface area, to hasten or accelerate chemical reactions. This catalytic property is common to many of the colloids but more especially to a class of substances known as enzymes which will be considered in a following chapter. The catalytic effect of finely divided substances is undoubtedly due to the enormous surface area exposed, which, by the process of adsorption, brings reactive materials into such close contact that reactions are stimulated.

Terms used in colloidal chemistry. The terms used in describing colloidal phenomena are unique to this branch of chemistry. It is necessary for the beginning student to master those expressions which are to be used in many subsequent chapters.

Each colloidal system has two different phases. The colloidal material, which is suspended or dispersed, is known as the *disperse phase*. The disperse phase, consisting of colloidal particles which are discontinuous, is distributed throughout a second medium known as the *continuous phase* or *dispersion medium*. The disperse phase and dispersion medium of colloidal chemistry correspond to the solute and the solvent of true solutions.

It should be emphasized at this point that it is not necessary to visualize solids in liquids as the only type of colloidal system. The table on p. 44 shows the different types of colloidal systems which are possible.

When a disperse phase has little affinity for the dispersion medium, the term *suspensoid* may be applied to the colloidal system, since such systems closely resemble suspensions. Suspensoids are referred to as *lyophobic systems* (solvent-hating) to indicate the lack of affinity of the disperse phase for the dispersion medium. If the dispersion medium of a lyophobic system is water, the system is said to be *hydrophobic* (water-hating). On the other hand, if a disperse phase has an affinity for a dispersion

COLLOIDAL SYSTEMS

Type	Term	Example
Solid in solid	Solid sol	Alloys, paper
Solid in liquid	Suspension	Paints
Solid in gas	Smoke	Iodine vapor
Liquid in solid	Gel	Glue, gelatin
Liquid in liquid	Emulsion	Blood, milk
Liquid in gas	Fog	Steam
Gas in solid	Solid foam	Pumice
Gas in liquid	Foam	Lather, froth
Gas in gas		(No example known)

medium, the colloidal system is said to be *lyophilic* (solvent-loving). When water is the dispersion medium of a lyophilic system, the colloidal system is said to be *hydrophilic* (water-loving). Lyophilic systems are called *emulsoids*. Emulsoids exist in two different forms. When the emulsoid takes the form of a liquid it is called a *sol*. An emulsoid which has the properties of a solid is referred to as a *gel*. Many sols will form gels upon heating. Such gelation is called *coagulation*. On standing, gels often contract, thus squeezing out a liquid. This process is called *syneresis*. The separation of serum from clotted blood is an example of syneresis. Many gels will swell when placed in water. This phenomenon of water uptake is known as *imbibition* and is often considered the opposite of syneresis.

It has been stated that the dispersed particles of a *stable* colloidal system do not settle out. The dispersed particles of stable colloidal systems possess either a positive or a negative electric charge. This electric charge may arise in one or more of several ways, e.g., (1) direct ionization of the substance constituting the disperse phase, or (2) the adsorption of an ion by the disperse phase. It is possible to determine the sign of the charge on a colloidal particle. When colloidal suspensions are subjected to the action of direct electric current, the colloidal particles migrate slowly to cathode or anode, depending on the charge they bear. The migration of charged particles in an electric field is called *electrophoresis*. By varying the hydrogen ion concentration of a colloidal system the charge on the colloidal particles can be removed. When this pH is attained, the particles will no longer migrate in an electrical field, and the colloidal

solution is said to be at its *isoelectric point*. At the isoelectric point colloidal solutions are most easily precipitated.

Precipitation of colloids. It has been shown that the stability of a colloidal solution depends upon electric charges on the dispersed particles. The neutralization or removal of these charges results in the precipitation of the colloidal particles. Such neutralization can be effected in the laboratory by mixing two colloidal sols having opposite charges. Thus, if a positive ferric hydroxide sol is added to a negative gold sol in proper proportion, the charges neutralize each other and both sols are precipitated. This is known as *mutual precipitation*. The addition of a salt solution to a sol will also cause its precipitation. In this case the ion having a charge opposite to that of the dispersed phase is responsible for the precipitation of the colloidal solution.

Hydrophilic systems (emulsoids) are far more difficult to precipitate than hydrophobic systems (suspensoids). This may be attributed to the fact that the stability of suspensoids is thought to be due solely to the charge on the particles, whereas the stability of emulsoids results from the charge on the particles and the affinity of the particles for the dispersion medium (water). The precipitation of an emulsoid, therefore, probably occurs in two stages: (1) the removal of the charge on the particles, and (2) the removal of water from the particles. The precipitation of albumin by the addition of $(\text{NH}_4)_2\text{SO}_4$ serves to illustrate the point. Although the first addition of $(\text{NH}_4)_2\text{SO}_4$ neutralizes the charge on the particles, the sol remains stable, owing to the hydrophilic nature of the albumin. The addition of more $(\text{NH}_4)_2\text{SO}_4$ causes the removal of water from the colloidal particles, until finally the stability of the solution is so affected that precipitation occurs.

4 • Carbohydrates

Continuing our discussion of the chemistry of plant and animal protoplasm, we shall now turn our attention to the various groups of carbon compounds (carbohydrates, fats, and proteins) which are of biological importance. When the plant puts forth its leaves and starts to "shift for itself" the first types of compounds formed in the leaves are the carbohydrates. For this reason, if for no other, we shall begin our discussion of carbon compounds by a brief consideration of this interesting group of substances.

In the early stages of chemical endeavor the chemist observed that the starches and sugars, when heated slowly in an open dish, charred to form carbon, giving off at the same time rather large amounts of water. Analyses of the sugars and starches revealed the fact that these compounds contained carbon, hydrogen, and oxygen, and that the latter were in proportion of two to one, as in water. It was natural therefore that these early workers should infer that the hydrogen and oxygen were combined as water in these compounds. In other words, it was thought that the starches and the sugars were hydrates of carbon; hence the name *carbohydrates*. Later investigation showed that, although hydrogen and oxygen were combined in the proportion of two to one, these compounds were not really hydrates at all. They were found to be aldehyde and ketone derivatives of polyhydric alcohols.

Although the starches and sugars are not the only substances classified under the heading of carbohydrates, they are by far the most important. For these it is possible to write a general formula $C_m(H_2O)_n$, which will apply to most of the members of this group. Recalling previous knowledge of organic chemistry, the student will remember that there are other compounds to

which the type formula mentioned above will apply. Examples of such compounds are acetic acid ($\text{C}_2\text{H}_4\text{O}_2$) and lactic acid ($\text{C}_3\text{H}_6\text{O}_3$). These acids follow the general formula for carbohydrates but are radically different from the sugars in their chemical structure and behavior. On the other hand, this general formula does not include special types of sugars referred to as the methyl sugars, of which rhamnose, a methyl pentose, is an example. *For these reasons it is better to define a carbohydrate as an aldehyde or ketone derivative of a polyhydric alcohol.* This definition eliminates the non-carbohydrate compounds but includes the methyl sugars.

GENERAL CHARACTERISTICS OF CARBOHYDRATES

Carbohydrates, particularly the simpler ones, are easily oxidized. They are, therefore, excellent reducing agents. It is to these compounds that most of the reducing properties of protoplasm are attributed.

Carbohydrates oxidize to form acids, indicating the presence



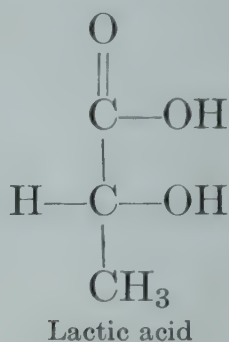
of either an aldehyde ($-\overset{\text{H}}{\underset{|}{\text{C}}}=\text{O}$) or a ketone ($=\text{C}=\text{O}$) group. The aldehyde group, upon reduction with hydrogen, forms the primary alcohol group ($-\text{CH}_2\text{OH}$); the ketone group forms a secondary alcohol radical ($-\text{CHOH}$). The polyhydric alcohols mentioned in a previous paragraph are organic compounds containing many alcohol ($-\text{OH}$) groups in the molecule. The simplest carbohydrates are, therefore, polyhydric alcohols containing either an aldehyde or a ketone group. Those containing the aldehyde group are called *aldoses*; those containing the ketone group are called *ketoses*. In most cases the ending *-ose* indicates that the substance is a sugar.

As can be seen from the definition of a carbohydrate, these substances contain a variety of organic functional groups or radicals. Recalling the general chemical characteristics of primary and secondary alcohols and of aldehydes and ketones, it is evident that even the simpler carbohydrates have the ability to enter into a large number of organic reactions.

Another general characteristic to be borne in mind is the fact

that carbohydrates are *amphoteric*; that is, they are capable of reacting as weak acids or weak bases. They will combine with stronger acids or bases to form salts.

Virtually all carbohydrates contain at least one asymmetric carbon atom, that is, a carbon atom to which four different groups



are attached. As can be seen in the formula for lactic acid, the central carbon has attached to it four distinctly unlike functional groups. Such a carbon is said to have *asymmetry*, hence the name *asymmetric carbon atom*. Any compound containing one or more asymmetric carbon atoms exhibits optical activity.

Optical activity. One very important property possessed by the carbohydrates is that of optical activity, which may be defined as the ability of a substance to rotate a plane of polarized light. Recalling his elementary physics the student will remember that a ray of ordinary light vibrates in all directions at right angles to the direction in which the ray is traveling. If such a ray of ordinary light is passed through a crystal of calcium carbonate (Iceland spar), it is split into two diverging rays. These rays vibrate in single planes at right angles to each other. They are said to be *plane polarized*. If a ray of ordinary light is passed through a *Nicol prism*, composed of two pieces of Iceland spar cemented together, one of the diverging rays is deflected. In this way it is possible to have only one ray of polarized light, vibrating in a single plane, emerge from such a prism. Many substances, of which the carbohydrates are excellent examples, have the power, when in solution, to rotate a plane of polarized light as it passes through the solution. Such compounds are said to be *optically active*. If the substance rotates the plane of polarized light to the right, it is said to be *dextrorotatory*. Conversely, if the plane is rotated to the left, the substance is said to be *levorotatory*. The degree of rotation is characteristic for any given substance and is proportional to the concentration of the sub-

stance in solution. The scientist has taken advantage of this fact and has developed an instrument for measuring the rotatory power of an optically active substance. Such an instrument is known as a *polariscope*.

In its simplest form a polariscope consists of two prisms, made of Iceland spar, situated in a metal tube, through which light may pass. The prism nearer the light source is in a fixed posi-

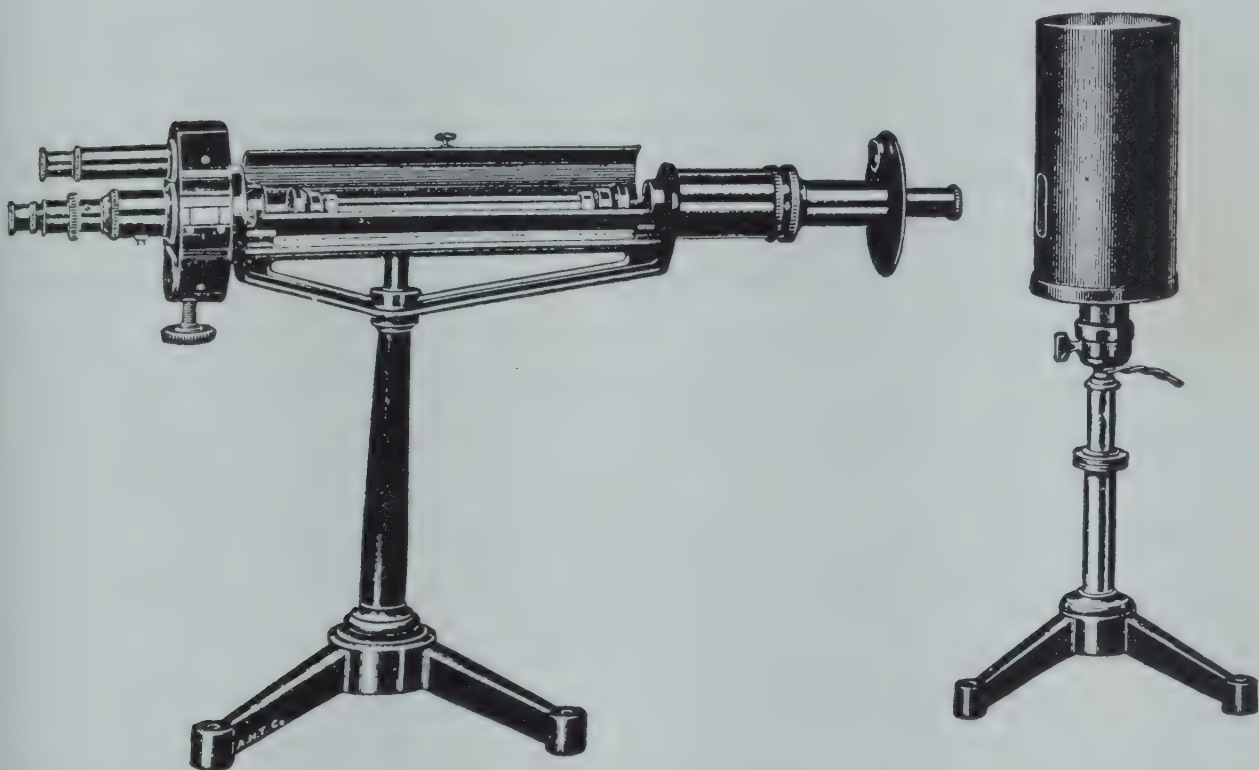


FIG. 4. A Schmidt and Haensch saccharimeter; a polariscope designed for the estimation of sugar. (Courtesy Arthur K. Anderson.)

tion and is called the *polarizing prism*. The Nicol prism at the opposite end of the tube can be rotated and is called the *analyzing prism*. When these prisms are oriented so that their optical axes fall in the same plane, the light that passes through the polarizing prism will also be transmitted through the analyzer without loss in intensity. Likewise, if the prisms are arranged so that their optical axes are at right angles to each other, no light will pass through the analyzing prism. In a polariscope the analyzing prism is arranged so that it may be rotated. A scale attached to this prism indicates the number of degrees through which it is rotated. Usually the zero point on the scale is at the point where the optical axes of the two prisms are at right angles to each other. When the analyzer is set at the zero point, no light will pass through the instrument. If at this point a solu-

tion of an optically active substance is placed between the prisms, the plane of light passing through the optically active substance is rotated either to the right or to the left. When this light strikes the analyzing prism some of it passes through because the plane of light is not at right angles to the optical axis of the analyzing prism. It is necessary, therefore, for the observer to turn or rotate the analyzing prism to the right or to the left until the light again disappears. If it is necessary to turn the analyzer to the right to accomplish this, the compound is said to be dextrorotatory. If, on the other hand, the analyzer is turned to the left, the solution is said to be levorotatory.

Optical isomerism. The optical activity of carbon compounds is due to the presence of one or more asymmetric carbon atoms in the molecule. We have already stated that lactic acid contains an asymmetric carbon atom and that it exhibits optical activity. When lactic acid is studied in the laboratory, two optically active forms are found. One of the forms is dextrorotatory, whereas the other is levorotatory. Both forms of lactic acid have the same empirical formula. They differ only with respect to their power to rotate the plane of polarized light. Compounds which have the same empirical formula but which differ in their behavior toward polarized light are said to be *optical isomers* or *stereoisomers*. This term refers to the geometrical arrangement of the atoms and atomic groups in space. This will be clear to the reader if we ask that he attempt to superimpose his left hand upon his friend's left hand with the palms of both hands facing in the same direction. It is found that they are not "isomeric" but identical, since they coincide, i.e., thumb over thumb, and finger over finger. If, however, he attempts to superimpose his own left hand on his own right hand, he will find that it is impossible to make them coincide unless he brings his hands palm upon palm. This shows, however, that his hands are not identical but that they are really mirror images.

Racemic mixtures. We have seen that there are two optically active stereoisomers of lactic acid, one dextrorotatory, the other levorotatory. A third variety of lactic acid is also known. This form is optically inactive; it is found in sour milk and is called *racemic* lactic acid. Racemic lactic acid is a mixture of 50 per

cent dextrolactic and 50 per cent levolactic acid. A 50:50 mixture of a dextro- and a levorotatory form of any substance is called a *racemic mixture* and is optically inactive, owing to the fact that the dextrorotatory power of one neutralizes the levorotatory power of the other. Such mixtures are said to be optically inactive because of *external compensation*.

Before actually entering into a detailed discussion of any of the carbohydrates, it is necessary for the student to have a basic knowledge of the nomenclature for these compounds.

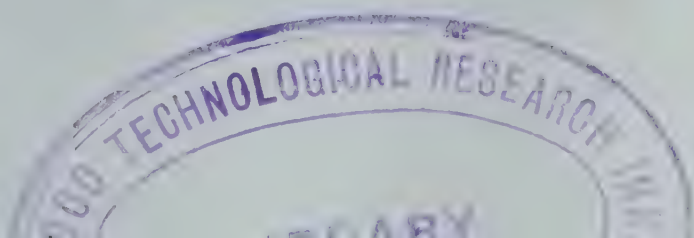
NOMENCLATURE

The carbohydrates, in general, have common names that end in *-ose*, such as sucrose, maltose, glucose, cellulose. The characteristic functional group, which is present actually or potentially, is the carbonyl ($=C=O$) group. In the aldoses this functional group is present as the aldehyde group; in the ketoses it is present as the ketone group.

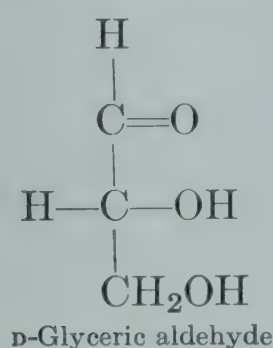
When the carbonyl group in the simple sugars is reduced with hydrogen, the corresponding polyhydroxy alcohols are obtained. The names of these polyhydroxy alcohols are characterized by the ending, *-itol*; thus arabinose upon reduction yields arabitol, and mannose yields mannitol. Oxidation of the functional carbonyl group results in the formation of an acid. The name of the acid is characterized by the suffix, *-onic acid*; thus glucose becomes gluconic acid, and mannose yields mannonic acid.

Authorities in the field of organic chemistry have suggested that carbohydrates should be named according to the International Rules of Nomenclature. These rules are the result of an attempt to standardize the naming of organic compounds throughout the world with only slight differences occurring due to different language requirements. Although this proposed nomenclature has many advantages, it has not as yet gained general recognition.

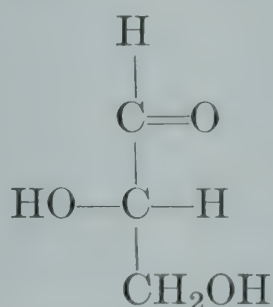
There is another important phase of nomenclature that must be stressed before the student can read intelligently in the field of carbohydrates. If he refers to the discussion of the different optical isomers of lactic acid, he will recall that there were two



such isomers. One turned the plane of polarized light to the right and was called dextrorotatory, and the other form turned the plane of polarized light to the left and was called levorotatory. This system of nomenclature, i.e., naming the compound according to its optical rotation, was carried over into carbohydrates. Accordingly dextroglucose was simply called *d*-glucose, levomannose was designated as *l*-mannose. As carbohydrate chemistry developed, more information accumulated concerning the true structural formulas of the sugars. All the structural formulas were related to that of the simple aldotriose, glyceric aldehyde. An examination of the formulas for the two isomeric

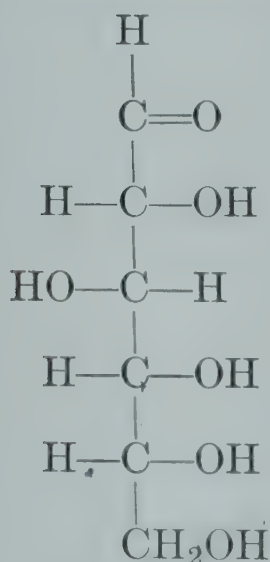
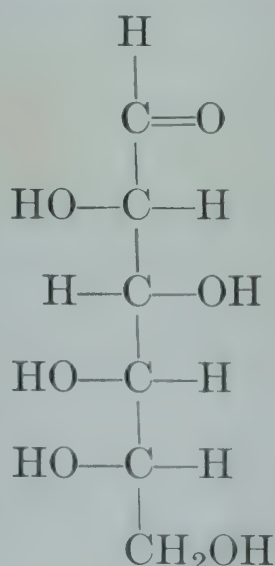


D-Glyceric aldehyde



L-Glyceric aldehyde

glyceric aldehyde molecules shows that the only difference between them is in the position of the hydroxyl group on the carbon next to the most reduced end of the molecule (i.e., next to the primary alcohol group). If, accordingly, the formula of a sugar is oriented so that the aldehyde group is at the top, the relative position of the OH group on the lower asymmetric carbon atom (i.e., the OH group on the carbon next to the most reduced end of the molecule) establishes the family relationship.

D⁺-GlucoseL⁻-Glucose

The formulas for the stereoisomeric forms of the aldohexose, glucose, will serve as an example of this relationship. A glance at the formulas shows that, in the case of D-glucose, the OH group on the carbon next to the most reduced end of the molecule is on the right side of the axis of symmetry. In L-glucose this same OH group is located on the left side of the carbon axis. Accordingly these sugars are named D-glucose and L-glucose, since they are related to D-glyceraldehyde and L-glyceraldehyde, respectively. It must be remembered that the terms D and L refer to *spacial* configuration and not to optical rotation. The rotatory power of a compound is designated by superscripts following the D or L family relationship. Thus, if a compound belongs to the D family and is dextrorotatory, we say it is D^+ . Conversely, if the D compound is levorotatory, it is designated as D^- . Examples showing the family relationship and the optical rotation are D^- -arabinose, L^- -glucose, D^- -idose, and D^+ -galactose.

By a discussion of the phenomenon of optical activity and with the introduction of the more important phases of the nomenclature of carbohydrates, we have attempted to lead the student into a deeper appreciation of the complexity of these compounds.

CLASSIFICATION OF IMPORTANT CARBOHYDRATES

For convenience of discussion and study it is advisable to classify carbohydrates. They are generally classified according to the types of compounds formed on hydrolysis. When a complex carbohydrate is completely hydrolyzed, the end products are organic compounds known as *monosaccharides*, *simple sugars*, or *one-unit sugars*. The monosaccharides serve as the "building blocks" for the more complex carbohydrates. For example, when two molecules of a simple sugar unite, with the evolution of one molecule of water, a compound known as a *disaccharide* is formed. It is evident, then, that disaccharides (two-unit sugars) will, upon hydrolysis, yield two molecules of a simple sugar. Three molecules of a one-unit sugar may unite to form a third class of carbohydrates known as *trisaccharides*, or *three-unit sugars*. Likewise, if a large number of simple sugars are

joined together, they form the most complex class of carbohydrates, known as the *polysaccharides* or *poly-unit sugars*.

Following is a classification of the more important carbohydrates:

CLASSIFICATION OF IMPORTANT CARBOHYDRATES

I. Monosaccharides (One-Unit Sugars)

A. Pentoses (five-carbon sugars) subdivided into:

1. Aldopentoses, of which arabinose, xylose, and ribose are the three most important members.
2. Ketopentoses, of which arabinulose is an example.

B. Hexoses (six-carbon sugars) subdivided into:

1. Aldohexoses, of which there are sixteen isomers, the most important being glucose, galactose, and mannose.
2. Ketohexoses, such as fructose.

II. Disaccharides (Two-Unit Sugars)

A. Sucrose. Anhydride of glucose and fructose.

B. Maltose. Anhydride of glucose and glucose.

C. Lactose. Anhydride of glucose and galactose.

III. Trisaccharides (Three-Unit Sugars)

A. Raffinose. Anhydride of glucose, fructose, and galactose.

B. Melezitose. Anhydride of two units of glucose and one of fructose.

IV. Polysaccharides (Poly-Unit Sugars)

A. Pentosans. Anhydrides of pentoses.

1. Arabans. Anhydrides of many arabinose units.
2. Xylans. Anhydrides of many xylose units.

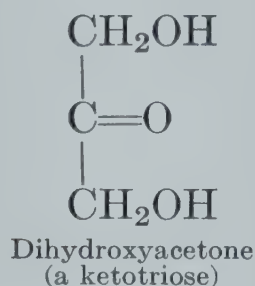
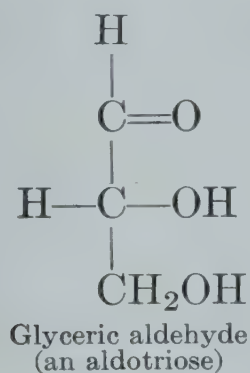
B. Hexosans. Anhydrides of hexoses.

1. Glucosans.
 - a. Starch. Anhydride of many glucose units.
 - b. Cellulose. Anhydride of many glucose units.
2. Fructosans (Levulans)
 - a. Inulin. Anhydride of many fructose units.
3. Mannans. Anhydrides of many mannose units.
4. Galactans. Anhydrides of many galactose units.

MONOSACCHARIDES

There are several types of monosaccharides or unit sugars also known as simple sugars. These are named according to the

number of carbon atoms in the molecule. For example, a *diose* sugar is a monosaccharide that contains two carbons. Theoretically, therefore, glycollic aldehyde, $\text{CH}_2\text{OH}\cdot\text{CHO}$, would be a simple diose sugar. By the same reasoning dihydroxy acetone, $\text{CH}_2\text{OH}\cdot\text{CO}\cdot\text{CH}_2\text{OH}$, one of the oxidation products of glycerol, is called a triose sugar. Glyceric aldehyde, $\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CHO}$, is likewise a member of the triose group.



A sugar containing four carbons would be called a tetrose, of which erythrose, $\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CHOH}\cdot\text{CHO}$, is an example. The pentoses and hexoses are carbohydrates containing five and six carbons, respectively.

The pentoses and the hexoses are the only monosaccharides of sufficient biological importance to merit detailed discussion.

Pentoses. As the name indicates, these are sugars containing five carbon atoms in the molecule. They are of some agricultural importance, although they cannot compare in importance with the hexoses. Although the pentoses do not generally occur as such in the plant, complex anhydrides of these materials are abundant in plants. From these polysaccharides the corresponding pentoses can be obtained by hydrolysis. A brief discussion of some of the more important pentoses follows:

D⁺-Xylose. This pentose is sometimes referred to as wood sugar. It can be prepared by the hydrolysis of wood, straw, seed hulls, and other fibrous material. Although this sugar is not of great commercial importance, it is used in bacteriological laboratories for the classification of bacteria.

D⁻-Arabinose. This sugar may be obtained by the hydrolysis of cherry gum, gum arabic, and mesquite gum. These gums are frequently found associated with pectin in plant materials. Recently small amounts of this sugar have been obtained from

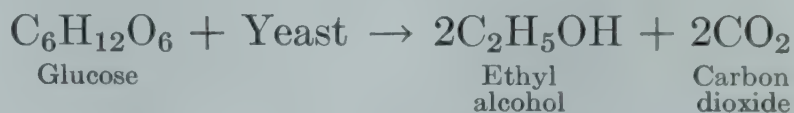
peas and beans. Arabinose is of very little commercial importance. Like xylose it is useful in the classification of bacteria.

D⁻-Ribose. This pentose is obtained as one of the hydrolytic products of nucleic acids. These nucleic acids will be discussed in another chapter. It has been found that most nucleic acids, on hydrolysis, yield either D⁻-ribose or a derivative of this sugar.

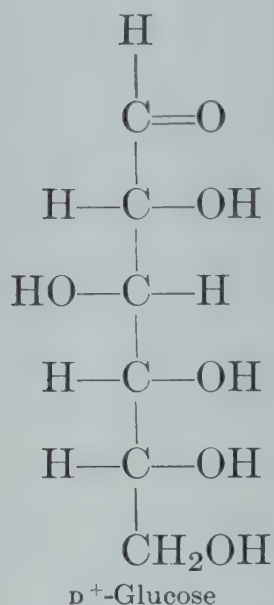
Hexoses. These sugars are of great importance because they are so widely distributed in nature. Three of them, D⁺-glucose, D⁻-fructose, and D⁺-galactose, will be discussed since they are the most important members of this group.

D⁺-Glucose. This sugar (also known as dextrose, grape sugar, starch sugar, or corn sugar) is undoubtedly the most important of the hexoses. It is found in such common materials as ripe fruit, sweet corn, onions, and honey. It is usually found associated with fructose, owing to the fact that these two sugars are formed when the disaccharide, sucrose, is hydrolyzed. Glucose is also formed when certain polysaccharides (glucosans) are hydrolyzed.

Glucose is sweet to the taste, but less so than sucrose (cane sugar). It is soluble in water and fermentable with yeast. On account of its structure, it is easily oxidized in the animal or plant organism. There is little question but that it is undoubtedly the most important transport form of sugar that we have. When glucose is subjected to the action of yeast, it is fermented, yielding ethyl alcohol and carbon dioxide.

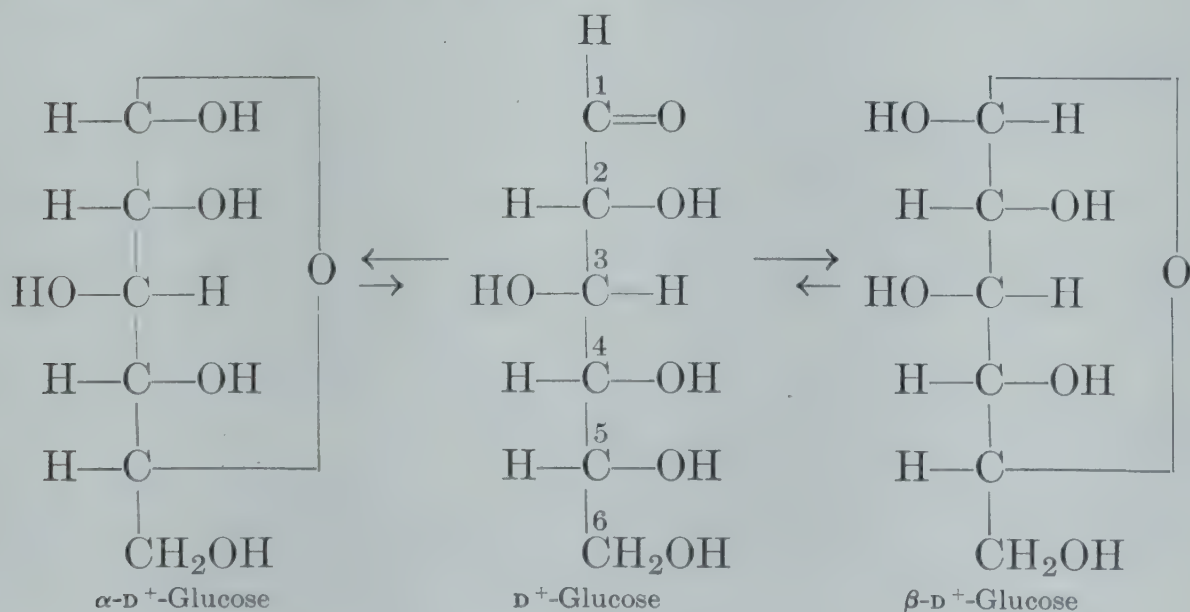


When glucose is allowed to react with acids, acid anhydrides, or acid chlorides, esters are formed in which five acid radicals combine to form a penta-ester. This indicates that there are five hydroxyl groups in the glucose molecule. The aldehyde group may be oxidized, in which case gluconic acid is formed. If the aldehyde group is reduced, the corresponding alcohol, sorbitol, is formed. These and other chemical reactions lead us to believe that glucose exists as a straight-chain compound and that its formula may be written as follows:



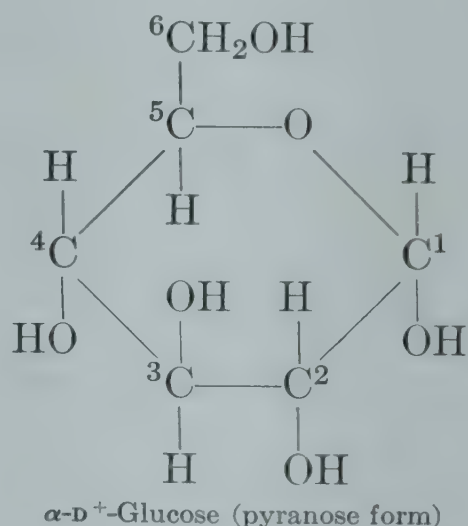
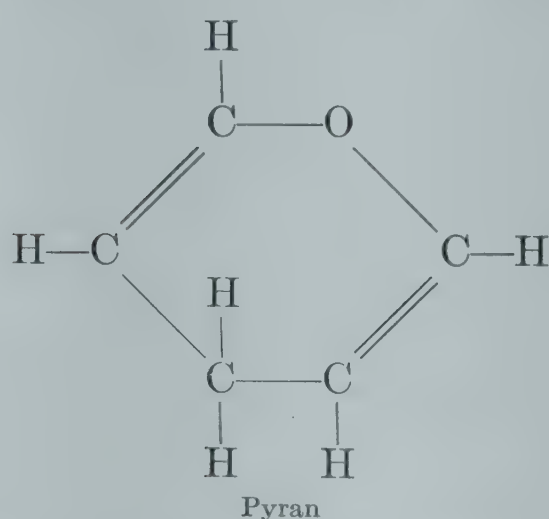
On the other hand, it is quite evident that certain chemical reactions that should occur (if this is the correct formula) do not do so. Furthermore, if one prepares a solution of glucose and observes its optical rotation, he finds that the rotation changes on standing. The rotation will change gradually over a period of a few hours and finally become constant. Such a change in rotation is called *mutarotation*.

This phenomenon of mutarotation seems to indicate that, when a sugar, such as D^+ -glucose, is dissolved in water, a change in the structure of the molecule takes place. This change in structure involves the aldehyde group (carbon 1) and the hydroxyl group on carbon 5. The hydrogen of this hydroxyl group migrates to the aldehyde group and, by breaking the double bond of the carbonyl radical, forms an OH group on carbon 1. Carbons 1 and 5 are then joined by the oxygen linkage, forming an oxygen bridge which is called an *amylen oxide ring*.



The formulas on p. 57 serve to illustrate this explanation. It will be noted that the above formulas differ only in the position of the newly formed OH group on the first carbon. When this hydroxyl group is on the right side of the carbon axis of symmetry, the compound is called alpha- (α -) glucose; conversely if the OH group is on the left, the compound is known as beta- (β -) glucose. It is thought that, when a sugar, such as D^+ -glucose, is dissolved in water, an equilibrium mixture is formed. The free aldehyde form is probably present in very small concentration, the bulk of the solution being composed of a mixture of the α and β forms. The aldehyde form of a sugar is often referred to as the active sugar. In spite of its low concentration it is this form of the sugar that actually enters into a chemical reaction, such as occurs with Fehling's solution. As the active sugar is removed in the process of a reaction, more of the active (aldehyde) sugar is formed from the α and β forms. Since the three forms of sugar are in equilibrium, this procedure is continued until the supply of the alpha and beta forms is exhausted.

Closer inspection of the formula for α - D^+ -glucose will show that this compound is, in reality, a heterocyclic aromatic compound. In other words the oxygen bridge completes a six-membered ring composed of carbons 1 to 5, with the oxygen as the sixth member. The relationship of this molecule to that of the heterocyclic compound, pyran, is readily recognized.

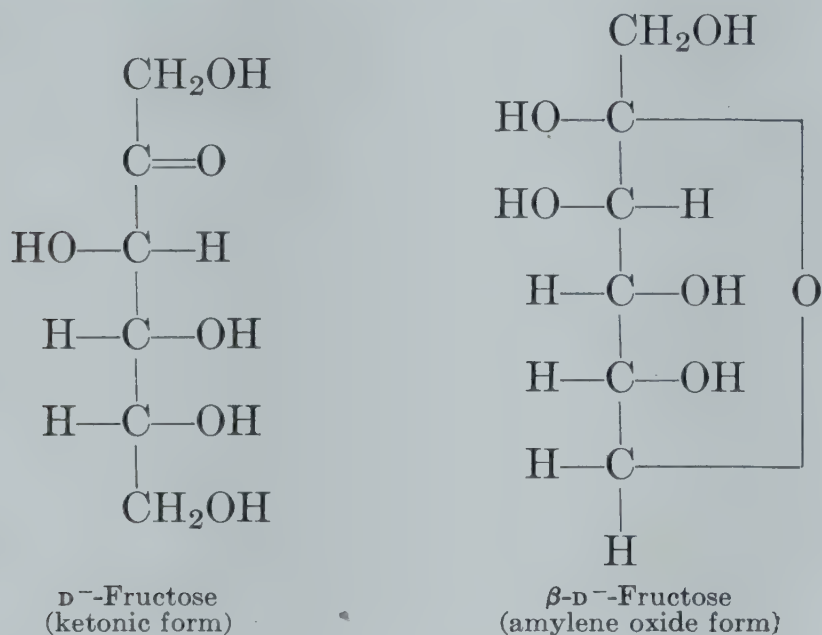


Thus, if we consider α - D^+ -glucose as a derivative of pyran, the formula is written as shown above.

D^- -Fructose. D^- -Fructose, levulose, or fruit sugar is found associated with glucose in honey and in sweet fruits. Fructose is strongly levorotatory, soluble in water, and much sweeter than

glucose. When cane sugar is hydrolyzed by acids or by enzyme action, glucose and fructose are found in equal amounts. Fructose may be prepared, uncontaminated with glucose, by hydrolyzing inulin, a polysaccharide obtained from dahlia bulbs and artichokes.

Whereas glucose is an aldohexose, fructose is characterized by the presence of a ketone group on the second carbon. Accordingly D^- -fructose may be assigned the following formula:



Fructose, like glucose, undergoes molecular rearrangement when dissolved in water. The amylene oxide form of fructose, containing the 2,6-oxygen bridge, is more stable than the more active ketonic form.

However, when fructose is combined with certain substances, it exists as a butylene oxide ring, characterized by a 2,5-oxygen bridge. Such a configuration is known as a *furanose* structure, and the corresponding compounds are called *furanosides*.

D^+ -Galactose. This hexose sugar does not occur free in nature but may be found in combination with other substances. Its most important source is the disaccharide lactose (milk sugar). When lactose is hydrolyzed, it yields D^+ -glucose and D^+ -galactose. Galactose is an aldehyde sugar less soluble than glucose and much less sweet to the taste.

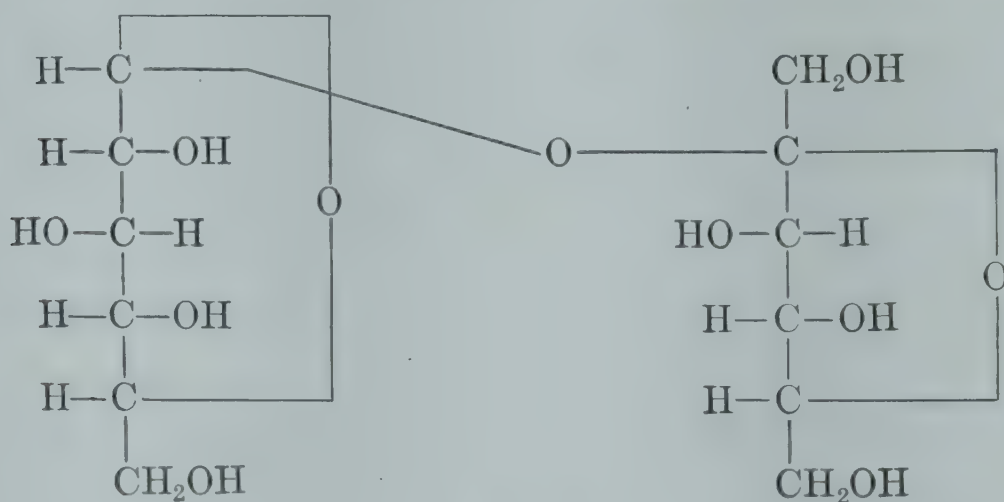
D^+ -Mannose. This sugar does not occur free in nature in appreciable amounts. It is best obtained by hydrolyzing the mannans, sometimes called the mannosans. Mannose is a dextro-rotatory aldohexose.

DISACCHARIDES

The disaccharides may be considered anhydrides of the monosaccharides. They are formed by uniting two molecules of monosaccharides with the evolution of one molecule of water. For this reason they are called two-unit sugars, since upon hydrolysis they yield two molecules of simple sugars. Only three naturally occurring disaccharides are of sufficient importance to merit discussion, namely, sucrose, maltose, and lactose.

Sucrose. This sugar is also known as cane sugar, beet sugar, and saccharose. It is the most important of the disaccharides and occurs widely distributed throughout the vegetable kingdom, usually associated with greater or lesser amounts of glucose and fructose. From the commercial standpoint the sugar cane, the sorghum cane, the sugar beet, the sugar maple, and the sugar palm are the best sources. In this country the largest amount of sucrose is obtained from the sugar cane and the sugar beet, the former containing as much as 20 per cent and latter from 10 to 25 per cent sucrose. Sucrose also occurs in varying quantities in the fruits and juices of many plants. For example, the pineapple contains about 11 per cent sucrose, and fruits like the strawberry, the apricot, ripe bananas, and apples usually contain about 5 to 6 per cent of this carbohydrate.

The carbohydrate content of the dilute nectar of flowers is composed almost entirely of sucrose. During the evaporation and concentration of this liquid by the honeybee, the sucrose is acted upon by enzymes which convert the sucrose to honey,



Sucrose
(1- α -D-glucose-2- β -D-fructose)

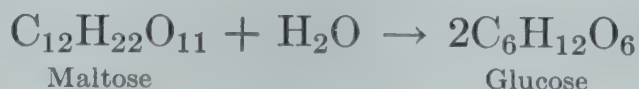
which is a mixture of glucose and fructose. The formula for sucrose is shown on p. 60.

It can be seen from the formula for sucrose that this sugar is composed of one molecule of α -D⁺-glucose and one molecule of β -D⁻-fructose. They are joined together through the potential aldehyde group (carbon 1) of the glucose and the potential ketone group (carbon 2) of the fructose. The possibility of the formation of α and β forms of the sugar is destroyed by this linkage. As a result, sucrose does not exhibit mutarotation.

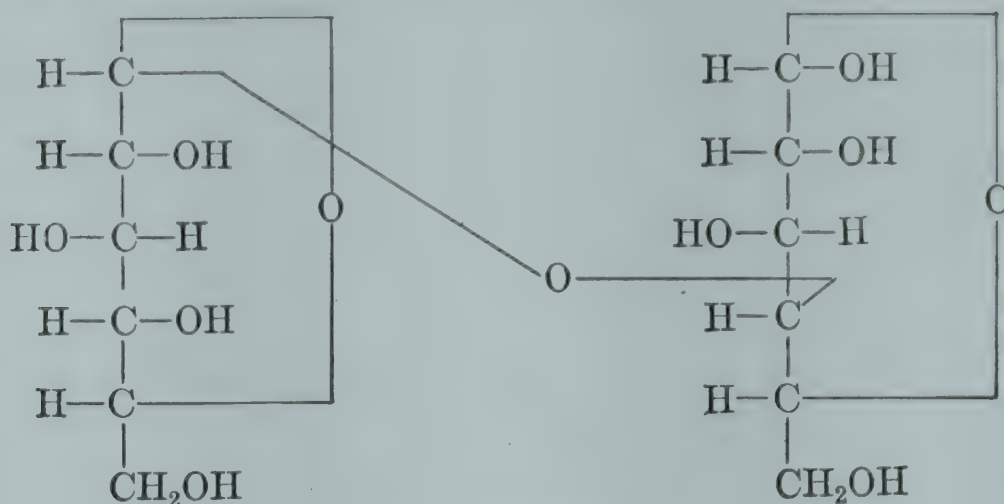
Because sucrose exists in a single form, it will crystallize readily. It forms large anhydrous crystals which are soluble in water. It is readily hydrolyzed by acids and by enzymes, called invertases. In water solution sucrose is quite strongly dextrorotatory, a property which becomes very useful in measuring the amount of this sugar in cane and beet juices at the factory. When dextrorotatory sucrose, which possesses a specific rotation of +66.5 degrees at 20° C, is hydrolyzed, the resulting mixture of sugars is found to be levorotatory. This is due to the fact that D⁻-fructose is strongly levorotatory, i.e., -93 degrees and more than counterbalances the dextrorotation of glucose (+52.6 degrees). Consequently the monosaccharides resulting from the sucrose hydrolysis have changed the optical rotation of the solution. This process is called *inversion*, and the resulting mixture of D⁺-glucose and D⁻-fructose is called *invert sugar*.

Maltose. Although this sugar possesses the same molecular formula as sucrose (C₁₂H₂₂O₁₁), it differs from sucrose in many of its properties. Maltose does not occur so abundantly in nature as sucrose. It is found in small concentrations in the cell sap of metabolizing plant tissues, and in larger concentrations in germinating cereals and malted grains when certain enzymes (amylases) exert their hydrolytic action on starch. For this reason maltose is also known as malt sugar.

Maltose is soluble in water and shows marked ability to reduce Fehling's solution. It is strongly dextrorotatory, having a specific rotation of +138 degrees. Maltose will not ferment until it has been hydrolyzed to its constituent monosaccharides. When hydrolysis occurs, maltose yields two molecules of glucose:



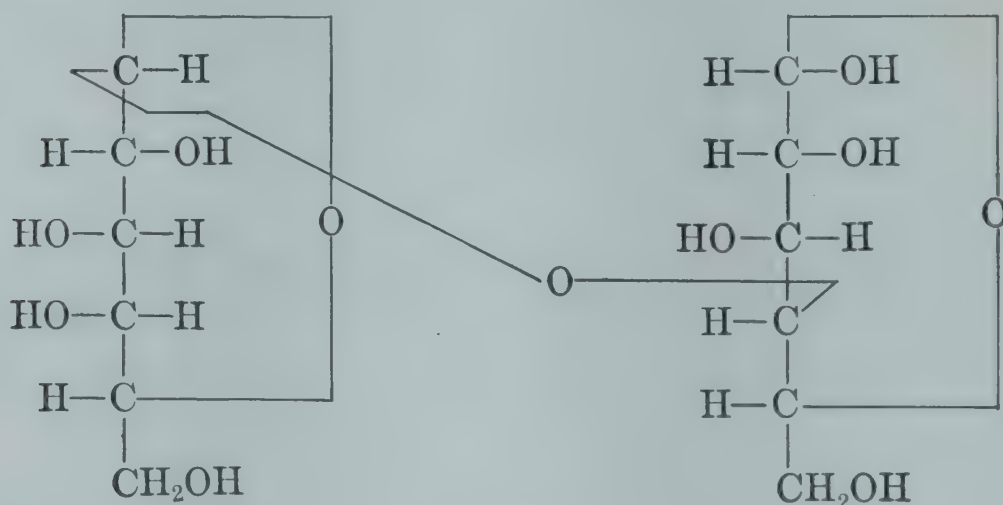
Maltose, like glucose, exhibits mutarotation in solution, forming α and β forms. Following is the formula for α -maltose, which is the most commonly occurring form.



α -Maltose
(1- α -D-glucose-4- α -D-glucose)

Lactose. This sugar is often called milk sugar. It occurs in the milk of all mammals, averaging about 6 per cent in human milk and about 4.8 per cent in cow's milk. Lactose appears to be synthesized in the mammary gland from the glucose carried in the blood to the gland. This sugar is not so soluble as sucrose and is much less sweet to the taste. On hydrolysis, lactose yields α -D⁺-glucose and β -D⁺-galactose.

Lactose has a specific rotation of +52.5 degrees. It exhibits mutarotation and, when in solution, exists in two forms, α and β . The α variety is not so soluble as the β form. Because of its greater solubility, β -lactose seems to be sweeter than the α form. As a result, β -lactose has found an important place in infant feeding. The formula for α -lactose follows:

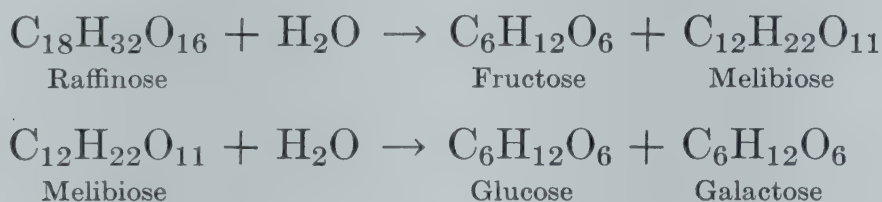


α -Lactose
(1- β -D-galactose-4- α -D-glucose)

TRISACCHARIDES

From an economic standpoint the trisaccharides are relatively unimportant. They are of scientific interest, however, since they are three-unit sugars which yield three monosaccharide molecules on hydrolysis.

The only member of this group that will be mentioned is *raffinose*. This sugar is found in small amounts in sugar beets, cottonseed, barley, and other plants. It is dextrorotatory, having a specific rotation of $+104$ degrees. Upon hydrolysis raffinose yields glucose, fructose, and galactose, the three important hexoses. This occurs in stages, however, as shown by the following equations:



Since raffinose is composed of the monosaccharides, glucose, fructose, and galactose, it is obvious that partial hydrolysis may yield the disaccharide, sucrose plus galactose, or another disaccharide, melibiose plus fructose, depending on the point in the raffinose molecule where the first hydrolytic cleavage happens to take place.

POLYSACCHARIDES

From the standpoint of plant economy, the polysaccharides are the most important of the carbohydrates. This is particularly true since these substances serve in the capacity of reserve carbohydrates in the plant and animal body. *Cellulose*, *starch*, and *inulin* are examples of the important plant polysaccharides, and *glycogen* is an example of the animal poly-unit sugar.

Chemically the polysaccharides are *anhydrides* of the simple sugars, since the latter may be formed from the former by the hydrolytic action of water. As a class the polysaccharides are colorless, odorless, and almost tasteless amorphous compounds. They are characterized by rather large molecules, although recent research has indicated that they may not be so complex as has been thought.

Instead of forming true solutions, which is characteristic of the sugars, most of these compounds form sticky or gelatinous solutions. It is for this reason that they are purified and studied with great difficulty. Owing to their colloidal nature it is most difficult to make accurate determinations of their molecular weights. For this reason authors usually write the empirical formulas for the pentosans $(C_5H_8O_4)_x$ and for the hexosans $(C_6H_{10}O_5)_x$ to indicate that the exact number of one-unit sugars in the polysaccharide molecule is not known. As has already been inferred, the polysaccharides yield, upon hydrolysis, many molecules of the simple sugars. Some polysaccharides yield pentoses and are known as *pentosans*. Others yield only hexose sugars and, consequently, are called *hexosans*.

Pentosans. The pentosans are substances of doubtful composition, existing in plant tissues. They may be isolated from such woody or fibrous portions of the plant, as straw, oat hulls, and corncobs. Although it is not possible to write structural formulas for the pentosans, they may be given the general formula $(C_5H_8O_4)_x$. Those pentosans which yield, on hydrolysis, the pentose sugar, *xylose*, are known as *xylans*. Xylans may be isolated from wood, straw, leaves, seeds, and vegetables. Those pentosans which hydrolyze to form the pentose sugar, *arabinose*, are called *arabans*. These compounds are isolated from gums, mucilaginous materials, and fruit juices.

Hexosans. From an agricultural standpoint this group of polysaccharides is undoubtedly the most important. The reason for this statement lies in the fact that the hexosans include the starches, glycogen, inulin, and cellulose, which are the most important storage forms of carbohydrates. These substances may be hydrolyzed to yield monosaccharides, all of which are hexose sugars. Those yielding glucose are called *glucosans*, whereas those yielding fructose are called *fructosans*.

Glucosans. This important type of carbohydrate occurs abundantly in nature in seeds, roots, bulbs, and tubers, and in the stems and leaves of most plants.

Starch. Although starches from different sources possess similar chemical properties, the physical properties are variable. In some plants, like the rice plant, the starch grains are small, whereas in others, like the potato, the starch grains are large and present a different appearance under the microscope. It is

by microscopic methods that the food and feed chemists are able to detect adulterations of one starch-containing food with another.

Starch grains do not dissolve in water but form colloidal suspensions. When such starch suspensions are heated to boiling, the granules swell, forming a colloidal solution which is usually called starch paste. When starch is treated with starch-splitting enzymes (amylases) or with boiling mineral acids, it is hydrolyzed to sugars. The hydrolytic action of enzymes is much more rapid on starch paste than on the raw starch granules. Although this is undoubtedly one of the important functions of cooking in the preparation of human food, research work has shown that most animals are capable of digesting large amounts of raw starch.

Starch is recognized as a mixture of two components called *amylose* and *amylopectin*. The *amylose* component is thought to be a straight-chain compound composed of about 100 to 700 glucose units joined between carbon 4 of one molecule and carbon 1 of the succeeding molecule. Amylose is readily dispersed in water and does not form the characteristic starch paste. Amylose will give the blue color with iodine.

Amylopectin is considered a branched chain consisting of 500 to 2000 glucose units. These units are joined to each other by the 4-1 glucosidic linkage, with the branches formed by a 6-1 glucosidic linkage occurring periodically in the chain. The amylopectin fraction is not readily dispersed in water and does form the characteristic starch paste. When amylopectin is treated with iodine, a purple or reddish color is produced.

Dextrins. When starch is hydrolyzed by enzymes, acid, or heat, a number of hydrolytic products are formed. Among these are the partially hydrolyzed starch molecules known as *dextrins*. These dextrins are less complex than starch and are more soluble in water, but the large dextrin molecules still retain many of the characteristics of starch. When these compounds are completely hydrolyzed they form maltose and finally glucose. The reader will note, therefore, that the final product of starch and dextrin hydrolysis is the monosaccharide, glucose. In physical properties and in appearance the high molecular weight dextrins are quite similar to starch, but they form sticky solutions with water which give them "gummy" properties. One commercial form,

known as British gum, is made by heating starch at 230 to 260° C. This form of dextrin is used extensively as an adhesive on paper labels and envelope flaps. A similar hydrolytic change occurs when starched clothes are ironed, the heated iron forming dextrin, which imparts stiffness to the fabric.

Glycogen. This is another hexosan that should be mentioned briefly at this point. This polysaccharide is the principal storage carbohydrate in the animal body and, on account of this fact, is sometimes called "animal starch." Glycogen is also a constituent of yeast and certain fungi. It is quite similar to the amylopectin fraction of starch in chemical and physical properties. It is a white, amorphous, tasteless powder which forms colloidal solutions and which is capable of producing glucose upon hydrolysis.

Glycogen is found in the lower forms of animals, such as the oyster, as well as in the higher animals. In the higher animals it is stored in relatively large amounts in the liver, although an appreciable amount may be found in the muscles and other tissues. The glycogen content of animal tissues is extremely variable. The reason for this lies in the fact that, if the animal has been active, glycogen is utilized so promptly after storage that the content of this substance is low. On the other hand, animals that are physically inactive may store large amounts of this polysaccharide.

Cellulose. This important group of polysaccharides belongs to the glucosans because glucose is the only monosaccharide formed on complete hydrolysis. Cellulose is the chief constituent of wood, the fibrous portions of plants, and the walls of most plant cells. The types of cellulose differ in toughness and strength, depending on the age and type of the plant. Wood fiber, cotton, and flax are good sources of cellulose. Cellulose is quite resistant to the action of most enzymes, dilute acids, and alkalies, and is digested with considerable difficulty by most animals, although ruminants and horses are able to utilize appreciable amounts. Books on feeding stuffs often refer to this material as "crude fiber" or the "indigestible carbohydrate" of the feed.

It is clear that, although cellulose does not constitute an important source of energy for man, it may serve as an important

source of energy for certain types of livestock. Aside from its value as a source of energy it plays an important role in the nutrition of all animals by serving as "bulk" and by facilitating the elimination of food residues. Cellulose exists in the purest form in cotton, where it appears as white amorphous fibers. It is insoluble in all ordinary solvents, but it can be made to form a thick solution when treated with Schweitzer's reagent, which is composed of copper ammonium hydroxide. The cotton can be reprecipitated from this solution by the addition of a strong mineral acid. Zinc chloride in solution is also a solvent for cellulose.

In woody tissues, such as we find in the wood of trees, cellulose is in combination with substances of unknown composition called *lignins*. Such combinations are known as *lignocelluloses*. In the manufacture of paper from wood, the wood is cooked in large digesters in the presence of water, sulfur dioxide, and lime. This removes the lignin, leaving the long cellulose fibers in the form of wood pulp, which is impure cellulose.

When cellulose is treated with alkali, it gelatinizes and the fibers become translucent, imparting to the finished product a silky luster. This product is known as *mercerized cotton*, since its discoverer was an Englishman named Mercer. Parchment paper is made by treating certain types of paper with sulfuric acid. Rayon is made from *viscose*, which, in turn, is made from cellulose by the action of sodium hydroxide and carbon disulfide. This viscous material is forced through tiny openings into an acid solution of sodium bisulfite which changes the sodium-cellulose compound back to cellulose again.

When cellulose is treated with nitric acid in the presence of sulfuric acid, cellulose esters or nitrates of cellulose are formed. Celluloid, gun cotton, and pyroxylin lacquers for automobiles are made from the nitrates of cellulose. Esters of cellulose and acetic acid also find a wide use in the manufacture of leather substitutes, moving-picture films, and airplane varnishes. The lower cellulose nitrates are dissolved in alcohol and ether and sold on the market as collodion or "new skin." The fact that cellulose forms esters with nitric acid and acetic acid shows that the molecule contains alcohol (hydroxyl) groups. Since cellulose also forms glucose on hydrolysis, its structure must be some-

what similar to that of starch. Like the sugars, cellulose has acidic properties, for it forms combinations with sodium, copper, and similar bases.

Fructosans. These polysaccharides form fructose on hydrolysis and are called *fructosans*.

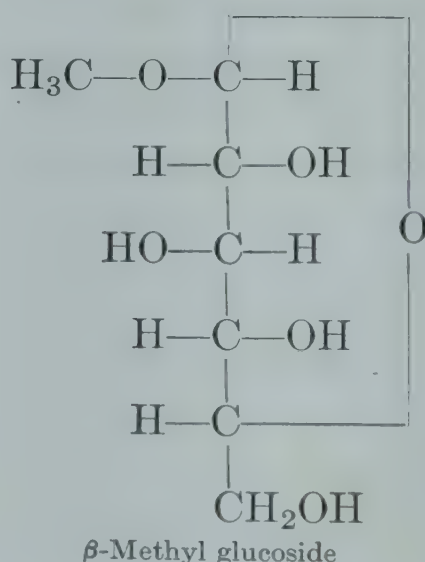
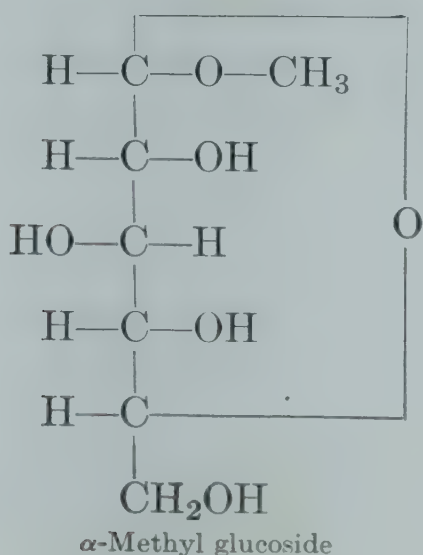
Inulin. Like starch and dextrin, inulin is a white, amorphous powder, which forms a colloidal suspension in hot water. Whereas the starches and dextrans rotate the plane of polarized light to the right, inulin is levorotatory. Inulin is found in commercial quantities in the Jerusalem artichoke tuber and in the bulbs of the dahlia plant.

COMPOUNDS ALLIED TO THE CARBOHYDRATES

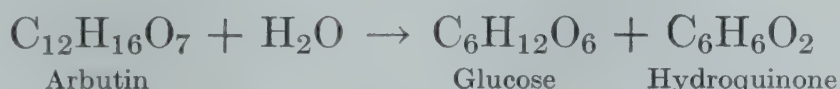
These compounds include a number of other carbohydrates or carbohydrate-like materials, the chemistry of which is not fully understood.

Glucosides. The term glucoside represents a group of compounds containing glucose and one or more basic or acidic organic compounds in chemical combination. Examples are known, however, where sugars other than glucose are present in the molecule. Such compounds are called *glycosides*.

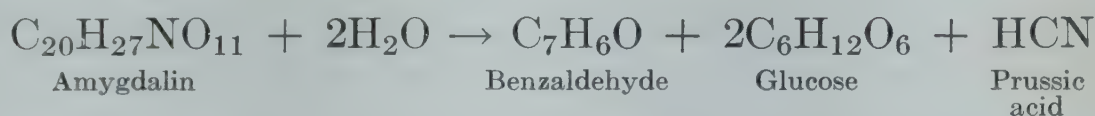
The glucosides are usually intensely bitter, colorless, crystalline compounds yielding levorotatory solutions. These compounds are readily hydrolyzed by acids and by certain enzymes. Glucosides may be classified as α - or β -glucosides, depending on the form of the sugar which combines with the non-sugar residue:



Many true glucosides have been isolated and identified, of which *arbutin* may be cited as an example. This compound, a normal constituent of pear leaves, yields the following products on hydrolysis:



Another well-known glucoside, *amygdalin*, is found in the kernel of peach and cherry seeds. On hydrolysis amygdalin yields the following products:



Pectins. Pectins are colloidal polysaccharides of high molecular weight and rather complex composition. On hydrolysis galacturonic acid, L-arabinose, D-galactose, methyl alcohol, and acetic acid have been obtained. Three pectin substances are known, namely, *protopectin*, *pectin*, and *pectic acid*.

Protopectin is the mother substance of this group of compounds. It occurs in the cell walls of plants and was formerly known as *pectose*. Protopectin can be hydrolyzed by the enzyme *protopectinase* into a soluble product known as pectin. Thus a transformation occurs during the ripening of fruits. Pectin contains more than 10 per cent of methyl alcohol combined as methoxy groups or as methyl ester groups. On removal of these groups by boiling with dilute acids, a compound is formed, known as *pectic acid*.

Mucilages. Mucilages are colloidal substances which appear to be sulfuric acid esters, where the ester group is a complex polysaccharide. Carrageen, or Irish moss, and agar-agar are among the best-known members of this group of compounds. On acid hydrolysis mucilages appear to yield galactose or an isomer of this sugar, mannose, and rhamnose (a methyl pentose).

REACTIONS OF CARBOHYDRATES

In a previous discussion of the general characteristics of carbohydrates we have pointed out that carbohydrates are characterized by the presence of either an aldehyde or ketone group

together with primary and secondary alcohol groups. Consequently these substances undergo a variety of reactions. Some of these reactions serve as a general test for carbohydrates as a class, whereas others are more specific for individual carbohydrates. A few representative examples of typical carbohydrate tests follow.

Molisch test. This is a general test for carbohydrates or for any compound containing a carbohydrate residue in the molecule. The test consists in mixing a few drops of α -naphthol with the solution to be tested, and carefully adding concentrated sulfuric acid so that it forms a layer at the bottom of the tube. The test is said to be positive when a violet color is formed at the junction of the two liquids.

Fehling's test. In a previous discussion it was pointed out that most carbohydrates are easily oxidized. Consequently these substances are excellent reducing agents. This fact is utilized by the chemist when he applies the Fehling test to a sugar. This test involves the use of two solutions known as Fehling's solution A and Fehling's solution B. Fehling's solution A is composed of copper sulfate, whereas solution B contains Rochelle salt (sodium potassium tartrate) and sodium or potassium hydroxide. Before the test is performed, these solutions are mixed in equal volumes. When a sugar solution containing a free aldehyde or ketone group is boiled with this solution, a brick-red precipitate of cuprous oxide (Cu_2O) is formed. The formation of this precipitate constitutes a positive test. Any sugar that reduces the cupric copper in Fehling's solution to the red cuprous oxide is said to be a *reducing sugar*. Only those sugars with a *free* or *potential aldehyde* or *ketone* group possess this characteristic reducing power. Examples of such sugars are glucose, fructose, maltose, and lactose. Sucrose, which does not contain these active groups, does not give a positive test with Fehling's solution.

Benedict's test. Benedict's solution differs from Fehling's solution in that Na_2CO_3 is substituted for the KOH , and sodium citrate is used instead of Rochelle salt. Benedict's solution is more sensitive than Fehling's solution.

Barfoed's test. When a sugar solution is heated in the presence of Barfoed's solution (cupric acetate in dilute acetic acid), the

cupric acetate is reduced to the characteristic brick-red precipitate, cuprous oxide. This reduction takes place quite rapidly with the monosaccharides, but very slowly with the disaccharides. This test can be used to distinguish between mono- and disaccharides.

Phenylhydrazine test. When phenylhydrazine ($\text{C}_6\text{H}_5\text{NH—NH}_2$) is added to a reducing sugar and the solution is heated, a yellow crystalline precipitate forms. This precipitate is called an *osazone* and is more or less characteristic of the sugar from which it is derived, with respect to crystalline form and melting point. Since this reaction involves the first two carbons of a sugar molecule, it is evident that all sugars with different spacial arrangements of H and OH groups on the remaining carbon atoms will yield different osazones. Likewise sugars that have identical spacial arrangements of H and OH groups on the remaining carbon atoms will form identical osazones. As an example, glucose, lactose, and maltose may be identified by the physical and chemical characteristics of their osazones. Glucose, fructose, and mannose on the other hand, yield the same osazone, since the configuration of carbons 3 to 6 are the same in each case.

5 • The Lipids

When living tissues are treated with ether, alcohol, petroleum ether, or chloroform, a number of substances are dissolved, among which are a characteristic group of compounds known as *lipids*. These consist of fats, oils, waxes, and sterols.

Although the various lipids are often unrelated chemically, they have a number of properties in common. First, as has been pointed out in the preceding paragraph, they are soluble in the so-called fat solvents; second, they are greasy or fatlike in their general properties; and third, they are relatively insoluble in water.

When lipids are classified they are usually divided into three main groups as follows: *simple lipids*, *compound lipids*, and their *hydrolytic products*. Simple lipids are defined as organic esters which, upon hydrolysis, yield only aliphatic alcohols and aliphatic monocarboxylic acids. On hydrolysis compound lipids yield aliphatic alcohols, aliphatic monocarboxylic acids, and another product, such as a carbohydrate, phosphoric acid, or an amine. The hydrolytic products of lipids are degradation products of the simple and compound classes and are therefore incapable of further hydrolysis.

CLASSIFICATION OF LIPIDS

- I. Simple lipids. Esters of aliphatic alcohols and aliphatic monocarboxylic acids.
 1. Fats and oils. Esters of glycerol and fatty acids. Fats differ from oils in that the former are solid at room temperature (20° C), whereas oils are liquid at the same temperature.
 2. Waxes. Neutral esters of fatty acids and relatively high molecular weight monohydric alcohols.

- II. Compound lipids. Esters of aliphatic alcohols and aliphatic monocarboxylic acids in combination with other compounds.
1. Phospholipids. Compounds composed of fatty acids, alcohol, phosphoric acid, and an amine.
 2. Glycolipids (cerebrosides, galactolipids). Compounds which upon hydrolysis yield a fatty acid, a carbohydrate (usually galactose), and a nitrogenous base.
- III. Hydrolytic products of lipids. This class includes the hydrolytic products of the simple and compound lipids.
1. Fatty acids.
 2. Alcohols (including glycerol and sterols).
 3. Miscellaneous compounds (including nitrogen bases and phosphoric acid).

FATTY ACIDS AND GLYCEROL

The compounds resulting from the hydrolysis of the simple lipids, fats, and oils, are the aliphatic monocarboxylic acids, called *fatty acids*, and the trihydric alcohol, *glycerol*. The physical and chemical characteristics of these degradation products merit emphasis and will be considered before continuing a discussion of their parent substances.

Fatty acids. Fatty acids are important constituents of all simple and compound lipids. Usually they are straight-chain aliphatic acids. Most of the members of this series contain an even number of carbons in the molecule. Thus the first member of the series, butyric acid, contains four carbons; the second member, caproic acid, contains six carbons in the molecule. Although the low molecular weight members of the series are found occasionally in natural products, they are not so important as the higher molecular weight acids such as palmitic acid ($C_{15}H_{31}COOH$) and stearic acid ($C_{17}H_{35}COOH$).

Fatty acids may be divided into four classes according to their molecular structure. These are: saturated, unsaturated, hydroxy-, and cyclic acids (see classification). Of these the saturated and unsaturated acids are most commonly present in natural products.

The saturated fatty acids in the series to and including the ten-carbon member, capric acid, are liquid at $20^{\circ}C$. Since they are volatile with steam, these acids are known as the *volatile*

CLASSIFICATION OF FATTY ACIDS PRESENT AS GLYCERIDES IN FATS

Name		Structural formula
Common	Systematic	
<i>I. Saturated Fatty Acids</i>		
A. Straight-Chain Series		
Butyric	Butanoic	$\text{CH}_3(\text{CH}_2)_2\text{COOH}$
Caproic	Hexanoic	$\text{CH}_3(\text{CH}_2)_4\text{COOH}$
Caprylic	Octanoic	$\text{CH}_3(\text{CH}_2)_6\text{COOH}$
Capric	Decanoic	$\text{CH}_3(\text{CH}_2)_8\text{COOH}$
Lauric	Dodecanoic	$\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$
Myristic	Tetradecanoic	$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$
Palmitic	Hexadecanoic	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$
Stearic	Octadecanoic	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$
Arachidic	Eicosanoic	$\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$
Behenic	Docosanoic	$\text{CH}_3(\text{CH}_2)_{20}\text{COOH}$
Lignoceric	Tetracosanoic	$\text{CH}_3(\text{CH}_2)_{22}\text{COOH}$
B. Branched-Chain Series		
Isobutyric	2-Methyl propanoic	$(\text{CH}_3)_2\text{CHCOOH}$
Isovaleric	3-Methyl butanoic	$\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{COOH}$
Tuberculoostearic	10-Methyl octadecanoic	$\text{CH}_3(\text{CH}_2)_6\text{CH}(\text{CH}_3)(\text{CH}_2)_8\text{COOH}$
<i>II. Unsaturated Fatty Acids</i>		
A. Oleic Acid Series		
1. Straight-chain		
Lauroleic	3-Dodecenoic	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CHCH}_2\text{COOH}$

CH₃(CH₂)₃CH=CH(CH₂)₇COOH
CH₃(CH₂)₇CH=CH(CH₂)₃COOH
CH₃(CH₂)₅CH=CH(CH₂)₇COOH
CH₃(CH₂)₇CH=CH(CH₂)₇COOH
CH₃(CH₂)₁₀CH=CH(CH₂)₄COOH
CH₃(CH₂)₅CH=CH(CH₂)₉COOH
CH₃(CH₂)₄CH=CH(CH₂)₁₀COOH
CH₃(CH₂)₉CH=CH(CH₂)₇COOH
CH₃(CH₂)₉CH=CH(CH₂)₉COOH
CH₃(CH₂)₇CH=CH(CH₂)₁₁COOH
CH₃(CH₂)₇CH=CH(CH₂)₁₃COOH

CH₃CH=C(CH₃)COOH

CH₃(CH₂)₄CH=CH—CH₂—CH=CH(CH₂)₇COOH

CH₃(CH₂)₇C≡C(CH₂)₇COOH

CH₃CH₂CH=CH—CH₂—CH=CH—CH₂—CH=CH(CH₂)₇COOH
CH₃(CH₂)₃CH=CH—CH=CH—CH=CH—CH=CH—CH=CH(CH₂)₇COOH

9-Tetradecenoic
5-Tetradecenoic
9-Hexadecenoic
9-Octadecenoic
6-Octadecenoic
11-Octadecenoic
12-Octadecenoic
9-Eicosenoic
11-Docosenoic
13-Docosenoic
15-Tetracosenoic

2-Methyl 2-butenic

9,12-Octadecadienoic

9-Octadecynoic

9,12,15-Octadecatrienoic

9,11,13-Octadecatrienoic

Myristoleic
Myristoleic
Palmitoleic
Oleic
Petroselinic
Vaccenic
Hapatic oleic
Gadoleic
Cetoleic
Erucic
Selacholeic
2. Branched-chain
Tiglic

B. Linolic Acid Series

1. Double bond

Linolic

2. Triple bond

Tariric

C. Linolenic Acid Series

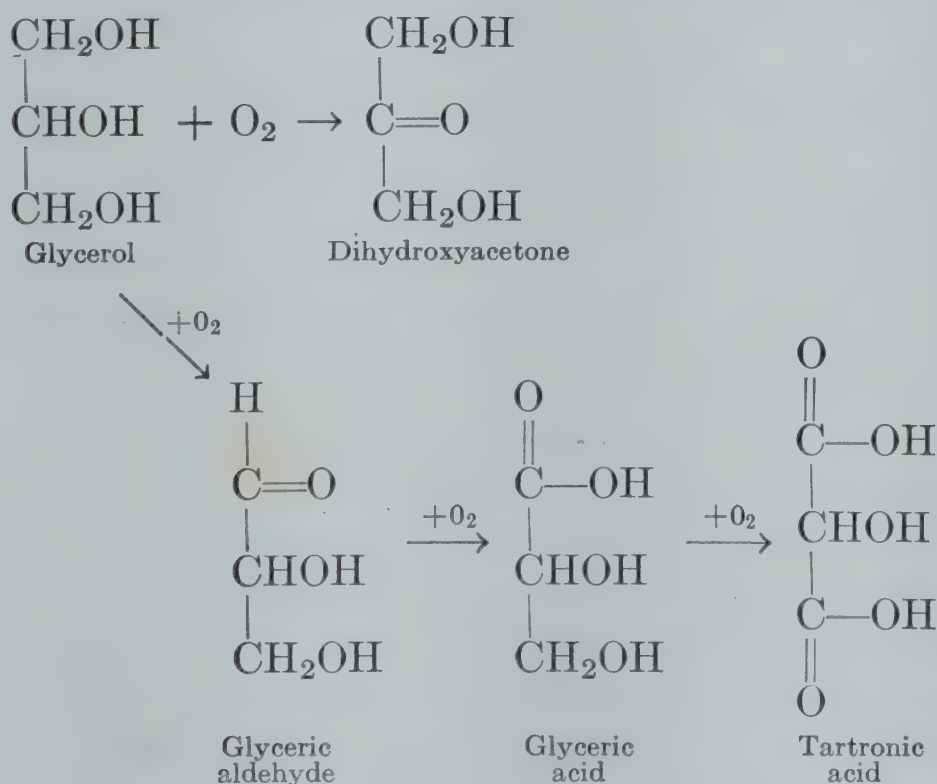
Linolenic

Eleostearic

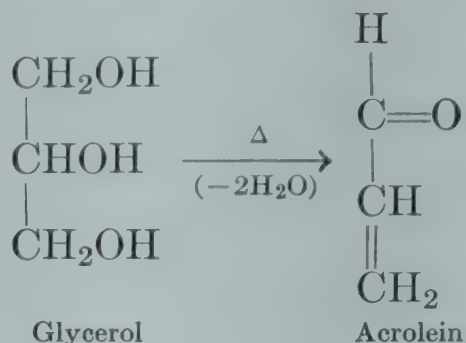
fatty acids. This property (volatility) is utilized in testing for the presence of these acids in such natural products as butter fat. Members of the saturated group of acids having more than ten carbons in the molecule are solid at 20° C and are not volatile with steam.

When a fatty acid has one or more double bonds in the molecule, it is said to be *unsaturated*. Generally speaking, the unsaturated fatty acids are of high molecular weight, having twelve or more carbons in the molecule. The predominant members of this class have eighteen carbons in the molecule. They are oleic acid (one double bond), linolic acid (two double bonds), and linolenic acid (three double bonds). The unsaturated linkages in these molecules are easily broken. As a result oxygen may enter the molecule and form an ethylene linkage which gives resinous properties to such fatty acids. When oils are rich in unsaturated acids of this type, they dry rapidly, owing to the absorption of oxygen at the double bonds. Such oils are called *drying oils*, of which linseed and tung oils are examples.

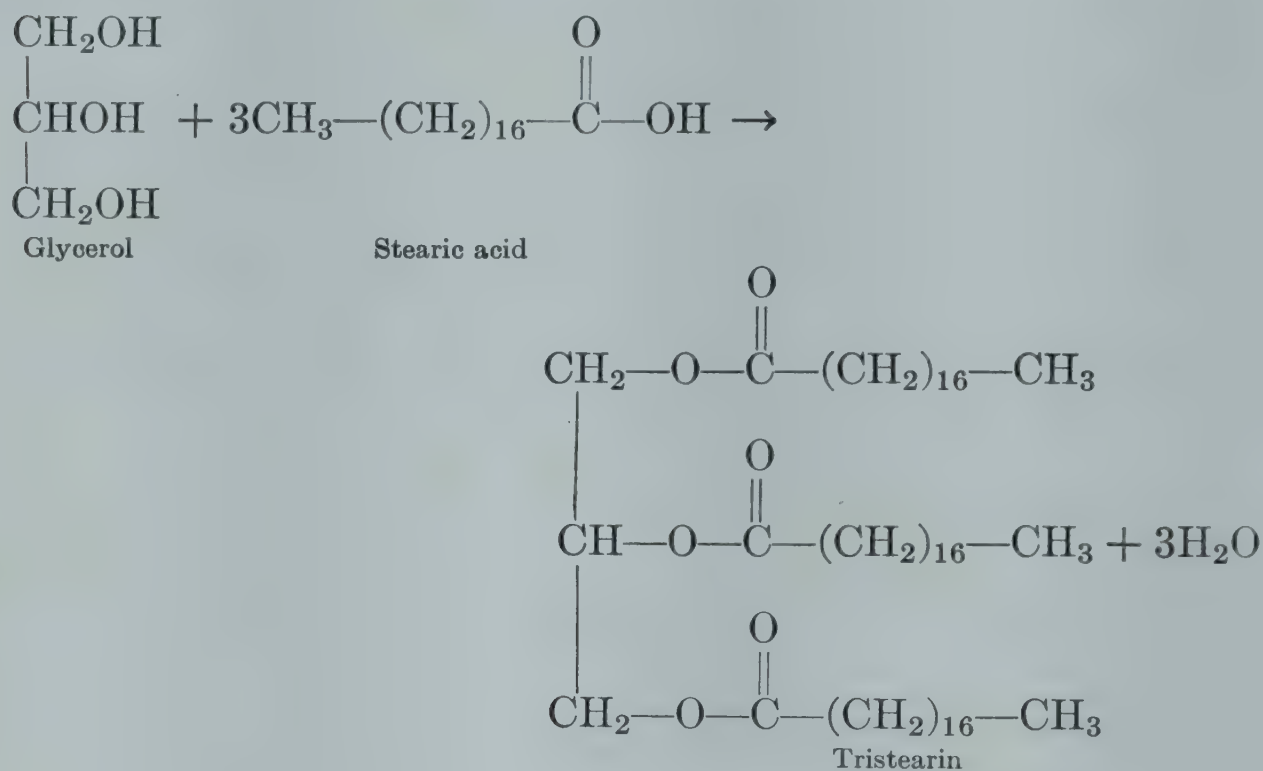
Glycerol. The trihydric alcohol, glycerol, is a common constituent of all fats and oils. Since glycerol is an alcohol containing primary and secondary alcohol groups, we may expect it to behave chemically in a manner similar to that of other primary or secondary alcohols. For example, by oxidation in easy stages we might expect, and in reality do obtain, the following products:



When glycerol is heated at high temperatures, as in the case of fats spilled on the top of a hot stove, water is removed and *acrylic aldehyde* (*acrolein*) is formed, a compound with a very pungent odor and irritating action.



Since glycerol is a trihydric alcohol, it has the ability to react with three molecules of a fatty acid to form a triple ester which is called a glyceride. Examples of such glycerides are the fats and oils. The following equation represents the formation of a glyceride:

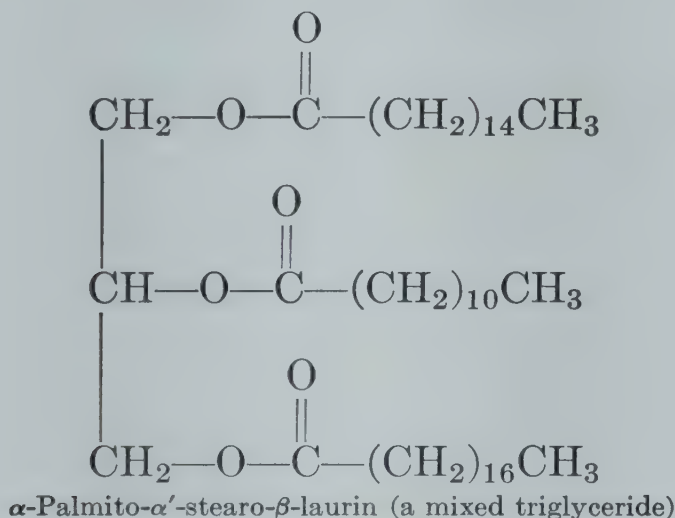


FATS AND OILS

From the standpoint of natural occurrence and commercial value, these simple lipids are the most important of all the groups included in the preceding classification. Although fats and oils differ in physical and chemical properties, they have so many characteristics in common that it is convenient to discuss them together. The fats and oils are characterized by their greasy

properties, and both types produce permanent translucent grease spots on paper. The main outstanding difference in physical properties lies in the fact that the fats are generally solid at room temperature, whereas the oils are liquid under similar conditions. Another generalization that can be made at this point is that saturated fatty acids predominate in fats, whereas oils contain rather large quantities of unsaturated acids.

Structure of fats and oils. Fats and oils are esters of glycerol and fatty acids. When three molecules of the same fatty acid combine with glycerol, as in the previous equation, the resulting compound is said to be a *simple glyceride*. If, however, glycerol combines with two or three molecules of different fatty acids, the glyceride formed is known as a *mixed glyceride*, of which α -palmito- α' -stearo- β -laurin, shown below, is an example.



In such a molecule the acids attached to the terminal carbons of the glycerol are said to be in the α position, and the fatty acid joined to the central carbon is in the β position. Physical properties, such as melting point and boiling point, vary with a change in position of a fatty acid in the molecule. Thus it is evident that a mixed triglyceride containing three different fatty acids can exist in three isomeric forms, each differing from the other in melting point, boiling point, and refractive index. Therefore *fats and oils are mixtures of mixed triglycerides* which vary in kind and amount in different types of fats and oils.

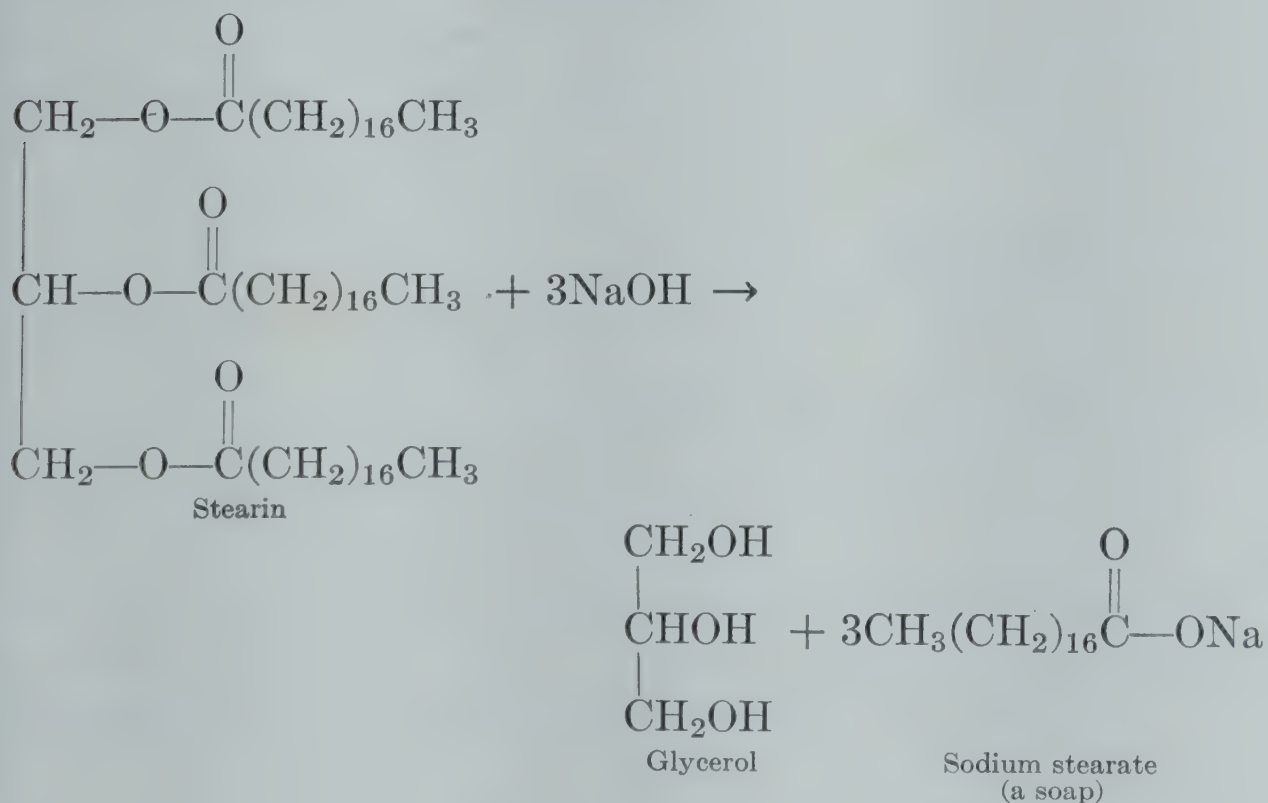
It is theoretically possible to have fats with one fatty acid or two fatty acids combined with glycerol. Such compounds are called *monoglycerides* and *diglycerides*, respectively. These compounds probably occur in small amounts in natural products.

Animal fats. Most of the simple lipids found in the tissues of warm-blooded animals are fats rather than oils. These animal fats vary in composition with different species of animals. However, the fat from an animal of any given species is more or less characteristic of that species. An examination of fat from different parts of the body of an animal shows marked differences in composition. For example, kidney fat of swine melts at 43°C ; lard, which is the body fat, melts at 28°C . Generally speaking, fats stored in fatty tissues as reserve materials are more saturated and have higher melting points than those located in the more active parts of the body. The fact that unsaturated fats are more easily oxidized than saturated fats seems to account for the presence of the former in the active tissues of the body, such as heart tissue, where the rate of metabolism is high.

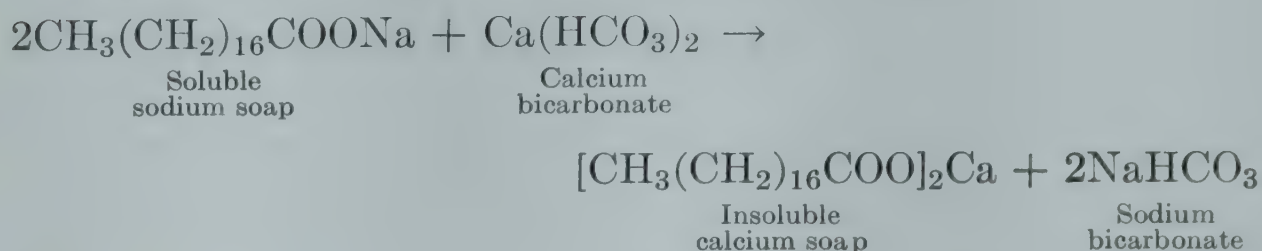
Vegetable oils. Glycerides are found in the vegetable kingdom as oils rather than as fats. Their liquidity is caused by the presence of a high percentage of unsaturated fatty acids in the glyceride molecules. The oxidation of the unsaturated fatty acids in the oil results in the ultimate formation of a tough resinous material known as "skin," a property of good "drying oils" used in paints. Oils may be classified according to their drying or "skin-forming" properties. Oils, such as tung oil and linseed oil harden rapidly on exposure to light and air and are said to be *drying oils*. Such oils contain relatively large quantities of linolic and linolenic acid and are very important in the manufacture of paints and varnishes where the resinous film is essential for the formation of the hard surface of the finished product. The absorption of oxygen in the formation of the resinous skin produces heat. This absorption of oxygen may become so rapid that sufficient heat is formed to bring the oil to its kindling temperature. This often happens when oil-soaked rags are thrown aside and forgotten. This process is known as *spontaneous combustion* and has been the cause of many disastrous fires. Oils, such as cottonseed oil, that harden slowly upon exposure to light and air are known as *semidrying oils*. Analysis of cottonseed oil shows that oleic and linolic acids are the predominant unsaturated acids present. Olive oil is an example of a *non-drying oil*. These oils do not harden upon exposure to light and air and are characterized by containing large quantities of oleic acid.

Hydrolysis of fats and oils. Since fats and oils are organic esters, they are easily hydrolyzed. Hydrolysis or splitting of fats and oils into their constituent parts may be accomplished by various means. Fats and oils will hydrolyze very slowly when intimately mixed with water. Hydrolysis is quite rapid when a fat is treated with steam. Another method of hydrolysis involves the use of Twitchell's reagent which consists of naphthalene, oleic acid, and sulfuric acid. When a fat or oil that has been preheated by steam comes into contact with small quantities of Twitchell's reagent, and the mixture is heated to the boiling point, hydrolysis occurs quite rapidly. Twitchell's reagent (and similar agents) are thought to hasten hydrolysis because they are somewhat soluble in fats and in water. They are thought to hasten the reaction through the formation of emulsions, thereby furnishing greater surface for the contact of the fat with the water. The use of such agents has become a commercial method for the hydrolysis of fats.

When fats are intimately mixed with an alcoholic solution of a strong alkali, such as sodium or potassium hydroxide, and are heated, the glyceride molecules are split, yielding glycerol and the sodium or potassium salts of the fatty acids. This process is called *saponification* (soap producing), and the metallic salts of the fatty acids are called *soaps*. The saponification reaction may be represented as follows:



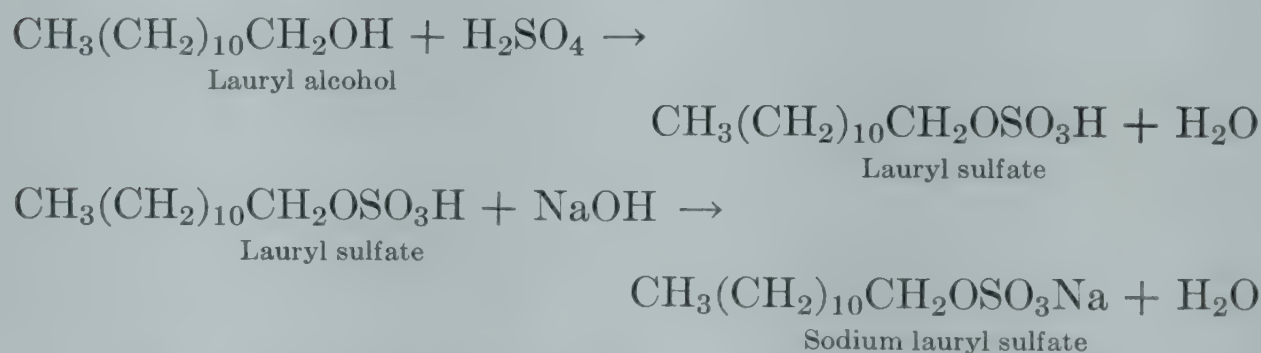
Soaps may be divided into two classes, i.e., *hard soaps* and *soft soaps*. Hard soaps, such as the common bar soaps, are the sodium salts of the higher fatty acids. Soft soaps are the potassium salts of the higher fatty acids and are usually marketed as a semiliquid or paste. The fatty acid salts of magnesium and calcium and the heavy metals, such as lead and zinc, are insoluble in water. When a sodium or potassium soap is added to "hard" water (water containing calcium or magnesium salts in solution) the sodium or potassium atom is replaced by calcium or magnesium, forming a soap insoluble in water:



The formation of such insoluble soaps explains the presence of "curds" when ordinary soaps are used in hard water.

Calcium soaps are used industrially as lubricating greases. Zinc soaps, especially zinc stearate, are used extensively in toilet powders. Lead, manganese, and cobalt soaps are used in paints to hasten the process of drying.

In recent years some products have been developed which serve as soap substitutes. They are prepared by reducing the higher fatty acids to the corresponding alcohols. These are treated with sulfuric acid and converted to the sulfuric acid esters and then to the corresponding sodium salts, as shown in the following equations:



The final product is a water-soluble substance with all the desirable characteristics of a soap. These *sulfated alcohols* are especially useful in hard-water areas because their calcium and

magnesium salts are water-soluble, thus eliminating the bothersome precipitation of the insoluble salts formed when true soaps are used. Examples of the sulfated alcohol type of detergent are the commercial products Dreft, Drene, and Gardinol.

Rancidity. Fats or oils, when exposed to light, air, heat, or moisture for an extended period of time, slowly develop off-flavors and bad odors and are said to be *rancid*. There are two principal types of fat rancidity, known as *hydrolytic* and *oxidative rancidity*.

Hydrolytic rancidity is produced as a result of the hydrolysis of fats and oils with the liberation of small amounts of one or more volatile fatty acids (butyric through capric). Dairy products are particularly affected by this type of spoilage. This is due to the presence of rather large amounts of the low molecular weight acids found in milk and milk products. The liberation of a trace of butyric acid, for example, will affect the odor and taste of the dairy products to the point where sales value is appreciably lowered.

Oxidative rancidity, from a fat economy standpoint, is the more important form of spoilage. In this type the unsaturated fatty acid fragments of the glyceride molecule are oxidized at their double bonds with the ultimate production of aldehydes, ketones, and acids with fewer carbon atoms in the molecule. The bad odor and objectionable taste of the rancid fat or oil are due to the presence of these decomposition products.

It has been known for a long time that different fats, when exposed to an atmosphere of pure oxygen, do not become rancid in the same length of time. Indeed certain fats are quite resistant to oxidation. However, when the initial resistance to oxidation is overcome, all fats absorb large quantities of oxygen with considerable rapidity and develop, with equal rapidity, the characteristic flavor and odor of rancidity. The length of time involved before a fat or oil begins to absorb large quantities of oxygen is known as the *induction period*. The induction period of a fat is of vital importance in so far as predicting its keeping quality is concerned. Fats with short induction periods cannot be stored for any length of time and consequently cannot be used in products where long storage is necessary.

The length of the induction period of a fat or oil seems to depend largely on the presence of small concentrations of substances other than the fat itself. These substances may be divided into two main classes: (1) those compounds which inhibit oxidation of the fat and (2) those compounds which accelerate the decomposition. The first group (those which inhibit oxidation) have been found to be compounds which are readily oxidized and which absorb large quantities of oxygen. A compound which prevents oxidation of a fat or oil and, in so doing, lengthens the induction period, is called an *antioxidant*. Such a substance will continue to inhibit the oxidation of the material it is protecting until the antioxidant itself is completely oxidized. Although the chemical nature of the majority of these antioxidants is unknown, it has been definitely established that ascorbic acid and α -tocopherol (vitamins C and E) have effective anti-oxygenic properties. Many other compounds have been shown to possess antioxygenic properties, but some of them cannot be used in food products because of their toxicity.

Opposing the action of the antioxidants in fats and oils is another group of compounds which accelerate the oxidation of the parent compound. Such substances are known as *pro-oxidants*, of which copper lactate is an example. Among the most noted pro-oxidants are the copper, iron, and nickel salts of organic compounds. It is generally believed that the majority of pro-oxidants are formed during the processing and refining of a fat or oil. Regardless of their origin, pro-oxidants are important factors to keep in mind when considering the keeping qualities of a fat or oil.

FAT ANALYSIS

It was mentioned in a previous paragraph that fats and oils are mixtures of mixed triglycerides. As a result even the most simple fats are complex mixtures of individual glyceride molecules. For example, if there are three different acids attached to the glycerol molecule, it has been shown that this gives rise to three different isomers, according to the position of attachment of these acids on the glycerol molecule. If four different

acids are present in the mixture, there are twelve theoretical isomers. Likewise, if five acids are involved, there are thirty possible theoretical mixed triglyceride isomers. Such a situation seriously complicates the problem of analysis, since no methods are available for the isolation of one isomer from another. Consequently fat analysis depends upon the collection of certain available data which are more or less characteristic and constant for each type of fat or oil. These determinations are called *fat constants*. The fat constants are *physical* and *chemical measurements* which seem to remain relatively constant for oils and fats prepared properly from the same source.

Physical constants. Among the physical constants most commonly determined are *refractive index*, *melting point*, *viscosity*, and *specific gravity*. The melting point, although not an extremely accurate determination, is a helpful guide in detecting adulteration of oils and fats and gives an approximate idea of the amount of unsaturated fatty acids present. Often, instead of determining the melting point of the fat, the chemist will determine the *solidification point* of the *free fatty acids*. This is done by saponifying the fat and treating the soaps with mineral acid. This liberates the mixture of the free fatty acids, which can be isolated and used to determine the solidification point.

Chemical constants. Among the chemical constants most commonly determined are *saponification number*, *iodine number*, and *Reichert-Meissl number*.

Saponification number. This constant yields very valuable information. The test is based on the fact that all fatty acids, regardless of molecular weight, are monobasic, each acid molecule uniting with but one potassium atom in the formation of soap. A known weight of the fat or oil is saponified with an excess of an alcoholic solution of potassium hydroxide of known strength. When the saponification reaction is complete, the excess alkali is determined by titration, and the amount of potassium hydroxide which united with the fatty acids is calculated. The *saponification number* is the amount of potassium hydroxide, expressed in milligrams, which is required to completely saponify 1 gram of fat or oil.

If the oil or fat is composed of acids of high molecular weight, such as stearic, palmitic, or oleic acid, there will be fewer molecules of fatty acids per gram of fat. Therefore the number of potassium ions in combination will be relatively few. The saponification number of a fat or oil, therefore, is an index of the relative size of the fatty acid molecules in the fat. Rapeseed oil contains appreciable quantities of erucic acid ($C_{22}H_{42}O_2$) and, as a result, has a saponification number of about 175. Oleomargarine averages about 195, since it contains relatively large amounts of the higher fatty acids. Butter fat, however, which contains appreciable amounts of acids of low molecular weight, such as butyric, caproic, and caprylic acids, has a saponification number that averages close to 227.

Iodine number. The degree of unsaturation of a fat or oil may be determined by allowing a known amount of fat or oil to react with solutions of known strength containing iodine-chloride (Wijs solution) or iodine-bromide (Hanus solution). The iodine will add to the double bonds of the unsaturated fatty acid fragments of the glyceride molecule, each double bond absorbing two atoms of iodine. Thus oleic, linolic, and linolenic acids will absorb two, four, and six atoms of iodine, respectively. By titrating the excess of uncombined iodine and calculating the amount absorbed, valuable information regarding the degree of unsaturation of a fat or oil may be obtained. *The iodine number* is defined as the *number of grams of iodine absorbed by 100 grams of a fat or oil*. For example, the iodine number of butter fat is from 28 to 40, whereas the iodine number of linseed oil is from 175 to 200. It is apparent from these figures that butter fat contains but few unsaturated acids, whereas linseed oil is highly unsaturated.

Reichert-Meissl number. This is an index of the amount of volatile fatty acids of low molecular weight. A known weight of fat or oil is saponified, and the soap is decomposed with dilute sulfuric acid, liberating the free fatty acids, which are subjected to distillation with live steam. Those containing from four to ten carbons are quite volatile with steam and may be removed by distillation and titrated with 0.1 N KOH. *The number of milliliters of 0.1 N KOH required to neutralize the volatile fatty*

acids in 5 grams of oil or fat is called the *Reichert-Meissl number*. Butter fat is an exception to the rule which states that naturally occurring fats and oils contain relatively small amounts of volatile fatty acids. Whereas the Reichert-Meissl numbers of coconut oil and palm oil range between 5 and 8, the value for butter fat ranges from 21 to 33. The naturally high Reichert-Meissl number of butter fat makes it possible to detect the presence of foreign fats and oils which are sometimes used as adulterants in the manufacture of butter.

WAXES

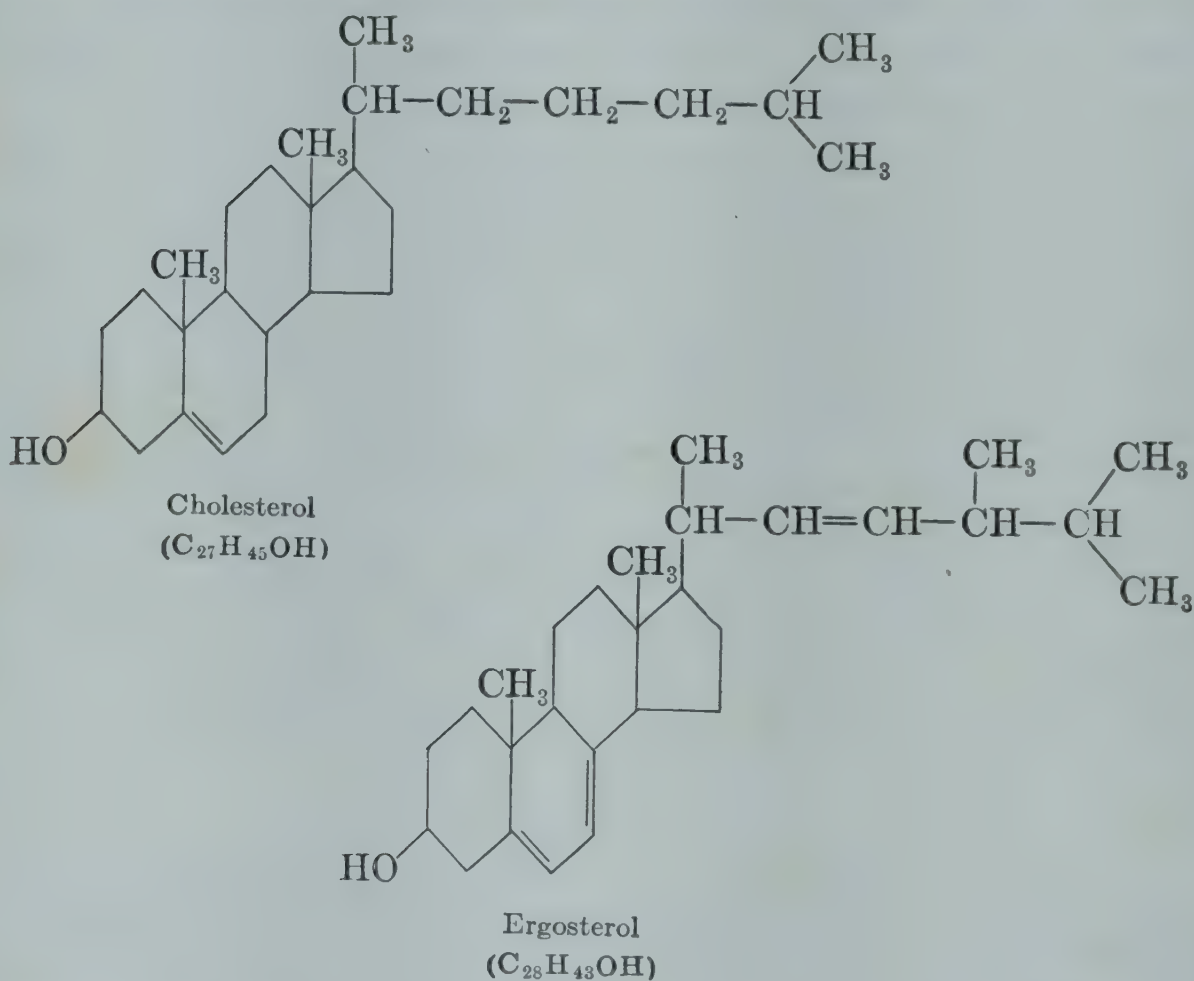
These compounds, like the fats and oils, are esters containing fatty acids. They differ from the glycerides, however, in that they contain high molecular weight alcohols instead of glycerol. In general, the alcohols are long-chain *monohydric alcohols*, such as cetyl alcohol ($C_{16}H_{33}OH$), melissyl alcohol ($C_{30}H_{61}OH$), and carnaubyl alcohol ($C_{24}H_{49}OH$). Sometimes the alcohol involved is *dihydric* as in the case of cocceryl alcohol ($C_{30}H_{60}(OH)_2$). Waxes may be animal or vegetable in origin, and their solubilities are similar to those of fats and oils. They may be saponified like the fats, but with much greater difficulty. The waxes occur quite extensively in nature, but as a rule they never occur abundantly. Beeswax, which is one of the best known of this group, contains myricyl alcohol ($C_{30}H_{62}OH$), combined with palmitic ($C_{16}H_{32}O_2$), cerotic ($C_{26}H_{52}O_2$), and melissic ($C_{30}H_{60}O_2$) acids. Waxes are found in thin layers covering the surfaces of the stems or stalks of many plants, where they function as a protective waterproof coating. The "bloom" on many fruits has been identified as a wax or a waxlike substance.

STEROLS

Although the word "sterol" literally means solid alcohol, the term has been limited in recent years to include only cyclic alcohols of high molecular weight. It has been shown that sterols are complex in structure, consisting of a nucleus closely related to the hydrocarbon, cyclopentanophenanthrene, and a side chain

of varying lengths and complexity, depending on the sterol in question.

Cholesterol is probably the best-known member of the group. It may be obtained in large amounts by extracting pulverized human gallstones with hot alcohol containing some potassium alcoholate. Cholesterol may be crystallized from alcoholic solution in the form of rhombic crystals, which, in chloroform solution, are levorotatory. When heated in vacuum at about 300°C , cholesterol may be distilled or sublimed without decomposition. It is easily dissolved in fats and in bile. On account of the fact that it is an alcohol and not an ester, it cannot be saponified; consequently it can be separated from fats following saponification by adding a suitable fat solvent such as ethyl ether. The water-soluble soaps remain in the water solution while the ether-soluble sterol is found in the supernatant ether layer. The chemical formulas for cholesterol and ergosterol are as follows:



Ergosterol. This sterol, the parent substance of one of the D vitamins, was first isolated from ergot, a fungus which grows

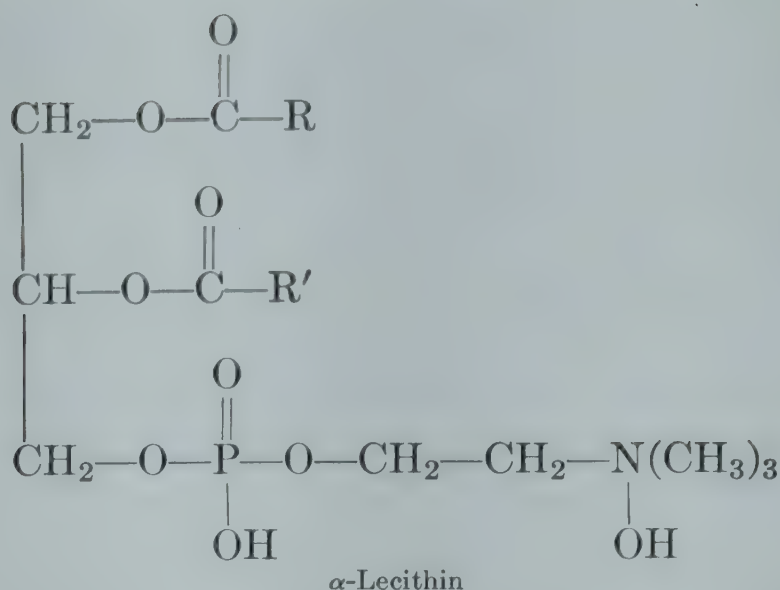
on plants, especially rye. This sterol has been found to exist in a number of food materials in amounts so small that its presence is difficult to detect. It occurs associated with phytosterol in plant tissues, and its separation is a matter of considerable difficulty. When exposed to ultraviolet light, ergosterol gives rise to several new compounds. One of these, calciferol, has been shown to possess antirachitic properties. In chemical composition ergosterol is not very different from cholesterol, although it contains three double bonds and, as a consequence, is much more unsaturated than cholesterol.

Phytosterol is a term generally applied to sterols found in plants. These sterols play an important part in the formation of plant waxes. Although a number of phytosterols exist, only a few have been differentiated. Among these are *sitosterol*, found in wheat embryo, barley, corn, and cottonseed, and *stigmasterol*, isolated from coconut, Calabar beans, and other plants.

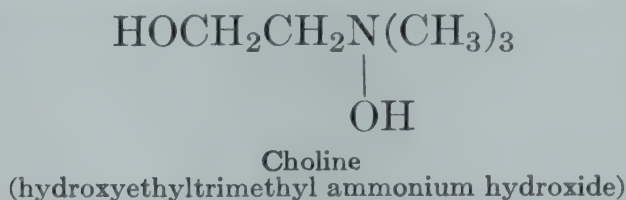
PHOSPHOLIPIDS

From a biological standpoint the phospholipids are one of the most important members of the lipid group. They are found in all plant and animal tissues and particularly in the most active tissues of the animal body such as the brain and liver. The properties of protoplasm, upon which all life processes depend, are intimately correlated with properties of the phospholipids.

The best-known members of the phospholipids are the *lecithins*. The lecithins are abundant in brain, liver, and nerve tissue of the animal, in the yolk of eggs, and in soybeans. Upon hydrolysis, the lecithins yield two molecules of fatty acids, glycerol, phosphoric acid, and a nitrogenous base, choline. It was generally believed that every lecithin contained one saturated and one unsaturated fatty acid radical in the molecule. Recent work, however, seems to indicate that both acids may be saturated, or both unsaturated, or one saturated and one unsaturated. It is probable that a large number of lecithins exist and that the number is limited only by the various binary combinations of the fatty acids. The following formula is representative of a typical lecithin:



The above formula represents a typical lecithin. R and R' are the fatty acid radicals which may be the same or different.



The nitrogenous base in this formula is choline, an important substance which acts as a regulator of fat metabolism.

GLYCOLIPIDS

These lipids upon hydrolysis yield galactose, a nitrogenous base, and a fatty acid. They are most commonly found in brain and nerve tissue. *Kerasin*, an example of this group of compounds, is found in the brain and yields, upon hydrolysis, galactose, a nitrogenous base, sphingosine ($\text{C}_{18}\text{H}_{33}(\text{OH})_2\text{NH}_2$), and lignoceric acid ($\text{C}_{23}\text{H}_{47}\text{COOH}$).

ESSENTIAL OR VOLATILE OILS

The classification of essential oils has always been a problem for writers of chemistry textbooks because these aromatic materials are related to no particular group or family of organic compounds and yet they are all related in one way or another to many groups of organic substances. For example, some volatile oils contain hydrocarbons which give them their characteristic properties; others contain alcohols, aldehydes, or esters.

Consequently some authors have classified them with the lipids for the reason that they are similar to certain lipids in some of their physical properties. We have not classified them with the lipids, but we feel that this is a logical point to discuss these interesting compounds.

All plant products owe their characteristic odor and taste to certain oils which differ in chemical composition and properties from the vegetable and animal oils just described. These aromatic oils are known as the *essential* or *volatile oils*.

Although it is probable that essential oils have no physiological function in the plant, they seem to be of service to many plants by attracting desirable insects, thereby aiding fertilization. It is equally evident that certain pungent essential oils seem to exert a repellent action toward certain insects.

Usually essential or volatile oils are insoluble in water. When fresh they are colorless or yellow in color, and most of them darken on exposure to air and light. Like the vegetable and animal oils they possess greasy properties and produce a translucent spot on paper. Unlike the glycerides of the fatty acids, however, the translucent spot is not permanent, owing to the volatile properties of these compounds. The essential oils are soluble in alcohol, ether, and chloroform, which is one reason why they are generally discussed with the lipids. Most of them can be distilled without change; this is not a characteristic of the fixed oils, which decompose prior to volatilization. Volatile oils are soluble in the fatty oils, and advantage is taken of this property in the manufacture of certain rare volatile oils, like the oil of rose.

Some volatile oils may be obtained from plant tissues by expression; some are separated by distillation with or without previous fermentation of the plant tissues; others, like attar of roses, are dissolved in a fatty oil and the mixture subsequently extracted and distilled. Some essential oils are extracted by volatile solvents which are later separated by fractional distillation.

Chemically the essential oils are so varied and heterogeneous that a satisfactory system of classification is difficult, although they may be classified roughly into four groups: *esters*, *aldehydes*, *ethers*, and *terpenes*. The methyl ester of salicylic acid, oil of

wintergreen, is an example of the first group. Cinnamic aldehyde, the principal constituent of *oil of cinnamon*, is representative of the aldehydes. *Oil of cloves* may be classified as an ether, since its principal constituent is *eugenol*, an aromatic ether. *Camphor*, *menthol*, and *oil of lemon* are examples of the terpenes.

6 • Proteins

Proteins are essential constituents of all living cells. The principal function of proteins in plants and animals seems to be in the regeneration and formation of tissues, although, like carbohydrates and lipids, they may be used in the production of energy.

Plants are able to synthesize these important chemical substances from simple nitrogenous compounds absorbed from the soil, and from water and carbon dioxide assimilated by roots and leaves, respectively. The animal, on the other hand, is not able to build these complex molecules from such simple materials. In general, animals synthesize these compounds from the digestion products of the proteins contained in their diet.

Since proteins are of such importance to animals, it has been customary for the farmer to grow many plants because of the commercial value of the proteins they contain. Feed manufacturers also produce animal foods, from plant and animal sources, which are rich in these important nitrogenous compounds. In later chapters we will learn that proteins do not all have the same value for the growing animal. This difference in the biological value of proteins lies in the fact that some proteins are rich in certain "building stones" called *amino acids*, whereas other proteins are devoid of some of these acids or contain them in very small amounts.

GENERAL PROPERTIES AND COMPOSITION OF PROTEINS

Nearly all proteins form sticky, colloidal solutions with water. For this reason it is most difficult to separate and purify a mixture of these compounds. However, methods of protein

separation have been evolved, based on their solubility in various reagents such as dilute salt solution, alkali, or alcohol. By using one or a combination of these solvents it is possible to extract a food material and separate the protein fractions. These fractions may be purified further by a series of recrystallizations or reprecipitations.

Analysis of such fractions shows that all proteins contain nitrogen, carbon, hydrogen, and oxygen. Most of them contain sulfur, and some of them, phosphorus. In a few cases other elements such as iron, copper, and manganese are present in the protein molecule.

In general, proteins have the following characteristics: (1) They are complex organic compounds of high molecular weight (from 15,000 to $>1,000,000$). (2) They are colloidal in nature and will not pass through membranes. (3) They are amphoteric; that is, they will act either as weak acids or weak bases. (4) They are hydrolyzed by acids, alkalies, or enzymes, forming a series of intermediate compounds, and eventually a hydrolyzate composed almost entirely of amino acids.

CLASSIFICATION OF PROTEINS

Proteins are numerous and very complex in structure, and, as a result, it is exceedingly difficult to classify these compounds. Nevertheless, a classification, no matter how imperfect, is essential for further discussion and study.

Essentially, proteins may be divided into three groups, namely the *simple*, the *conjugated*, and the *derived* classes. A *simple protein* is defined as one that will yield only amino acids upon complete hydrolysis. (Strictly this is not correct, since many of the albumins contain small concentrations of a carbohydrate such as galactose.) Simple proteins are subdivided into seven groups, according to their solubility in various reagents. *Conjugated proteins* are complex compounds in which the protein is associated with some other molecule such as a carbohydrate, a lipid, or a color-bearing substance. The subdivisions of this group are made according to the type of molecule or molecules with which the protein fragment is conjugated.

When simple and conjugated proteins are exposed to heat, water, enzymes, or certain chemical reagents, the third class, the *derived proteins*, is formed. The derived proteins, which may be considered decomposition products of the simple and conjugated classes, are subdivided into two groups, the *primary derived proteins* and the *secondary derived proteins*. The classification of a derived protein into one of these groups depends upon the extent to which decomposition has taken place. Primary derived proteins differ but slightly from the original protein molecule, whereas secondary derived proteins have been appreciably affected by heat, water, enzymes, or chemical reagents.

A. Simple proteins. Compounds occurring in nature and yielding on hydrolysis chiefly α -amino acids or their immediate derivatives.

1. *Albumins*. Soluble in water and neutral salt solutions; coagulable by heat; salted out by saturation with ammonium sulfate but not by saturation with sodium chloride except in the presence of acid; give all protein color and precipitation tests and usually lack none of the indispensable amino acids among their hydrolysis products; examples are ovalbumin from white of egg and serum albumin from blood plasma.

2. *Globulins*. Insoluble in pure water and very dilute salt solutions; soluble in 1 per cent or slightly stronger solutions of neutral salts; coagulable by heat; salted out by half saturation with ammonium sulfate and by complete saturation with sodium chloride; give all protein color and precipitation tests and usually lack none of the indispensable amino acids among their decomposition products; examples are ovoglobulin from white of egg, serum globulin from blood plasma, edestin from hempseed, many globulins similar to edestin from other seeds and nuts, and myosin from meat.

3. *Glutelins*. Insoluble in water or neutral salt solutions, but soluble in very dilute acids or alkalies; assume a sticky, tenacious, gel-like condition upon imbibition of water as in dough-making; coagulable by heat; show no conspicuous lack of any amino acid; examples are glutenin from wheat and oryzenin from rice.

4. *Prolamines*. Insoluble in all watery solutions, but soluble in 60 to 80 per cent alcohol; not heat-coagulable; contain the

largest amounts of proline found in any proteins, amounting to 10 per cent or more of the molecule, also the largest amount of glutamic acid found in any proteins, amounting to 43 per cent in one case; examples are gliadin from wheat, hordein from barley, secalin from rye, zein from maize, and others from various seeds.

5. *Albuminoids or scleroproteins*. Insoluble in all reagents which do not decompose them; digested slowly and with difficulty, if at all, by all gastrointestinal enzymes; tend to yield a disproportionately large amount of simpler amino acids such as glycine, and are deficient or entirely lacking in one or more of the more complex amino acids such as tyrosine and tryptophan; examples are keratin from epidermis, horns, hair, wool, nails, and other skin appendages; collagen from bones, tendons, and other connective tissues; and fibroin of silk.

6. *Histones*. Soluble in water and dilute acid solutions; insoluble in ammonia; soluble in sodium or potassium hydroxide; not coagulated by heat; predominantly basic in character and yield comparatively large amounts of diamino acids; occur in nature as components of compound proteins; examples are globin (denatured) of hemoglobin from blood and histones of nucleoproteins from various plant and animal tissues.

7. *Protamines*. Simple proteins of comparatively low molecular weight; soluble in water; dilute acids and alkalies (including ammonia) not coagulated by heat, so predominantly basic that their watery solutions are alkaline to litmus; combine with large proportions of acid but have only a slight combining power for alkalies; composed largely of diamino acids, especially arginine; occur in combination with nucleic acid in the heads of spermatozoa; examples are salmin from salmon sperm, sturin from sturgeon sperm, clupein from herring sperm, and several others that have been prepared from various kinds of fish sperm.

B. Compound proteins. Substances occurring in nature and yielding on hydrolysis, in addition to α -amino acids, some non-protein group, sometimes called the *prosthetic group*.

1. *Nucleoproteins*. Yield nucleic acid; occur most abundantly in cell nuclei but not confined to them; are generally combina-

tions of a histone with nucleic acid; examples are thymus nucleoprotein and yeast nucleoprotein.

2. *Chromoproteins*. Colored proteins, composed of a histone united to a color group containing a metal; include the respiratory pigment proteins of blood; examples are hemoglobin from blood and hemocyanin from invertebrate blood.

3. *Glycoproteins*. Yield sugarlike substances as the prosthetic group; examples are mucin from saliva and mucoid from connective tissues.

4. *Lecithoproteins*. Yield lecithin (phosphorized fat) as the prosthetic group; have not been sufficiently studied to make certain whether they actually occur in nature or are formed during the process of their preparation from various plant and animal substances, for example in egg yolk.

5. *Lipoproteins*. Yield fatty acid as the prosthetic group, and, like lecithoproteins, have not been definitely proved to occur in nature but may exist in all plant and animal tissues.

6. *Phosphoproteins*. Sometimes classed as simple proteins since no organic prosthetic group has been identified; hydrolyze to yield phosphoric acid which has been regarded as the prosthetic group; are predominantly acid in character; important in nutrition of growing animals; examples are casein from milk and ovovitellin from egg yolk.

C. Derived proteins. Proteins obtained by partial hydrolysis or by denaturation of natural proteins. Some of them are intermediary products of protein hydrolysis. The group also includes the slightly modified (probably not hydrolyzed) proteins obtained by heat coagulation and the synthetic substances called peptides.

1. *Coagulated proteins*. Produced by heat or alcohol coagulation, which is apparently a process of dehydration; insoluble in all reagents that do not decompose them.

2. *Metaproteins or infraproteins*. Produced by brief action of dilute acids or alkalies on natural proteins at temperatures below boiling; examples are proteins formed by action of dilute acid on certain globulins at room temperature, alkali metaprotein formed by action of dilute NaOH or KOH on natural proteins

at 30 to 60° C, and acid metaprotein formed by action of dilute acid on natural proteins at 30 to 60° C.

3. *Proteoses*. Produced by action of dilute acids or protein-digesting enzymes when hydrolysis is permitted to go beyond the metaprotein stage; divided into primary and secondary proteoses; the primary proteoses are salted out by half saturation with ammonium sulfate and are precipitated by nitric acid and by picric acid, whereas secondary proteoses are salted out only by complete saturation with ammonium sulfate and are not precipitated by nitric or picric acid; secondary proteoses have a smaller molecular weight than primary proteoses and represent a more advanced stage in the hydrolytic cleavage of natural proteins; proteoses are not coagulable by heat; many of them are powerfully toxic when injected into animals; example is albumose from albumin.

4. *Peptones*. Produced by action of dilute acids or protein-digesting enzymes when hydrolysis is permitted to go beyond the proteose stage; cannot be salted out by ammonium sulfate or any other salt; are not precipitated by nitric acid or picric acid; have a molecular weight small in comparison with natural proteins; in complexity and general chemical behavior resemble very closely the artificially synthesized polypeptides, which may, indeed, be defined as peptones of known molecular structure.

Amino acids. When proteins are hydrolyzed by means of acids or alkalies, the sticky colloidal properties disappear and the proteins go into solution. Similar changes take place when protein-digesting enzymes are allowed to react with protein suspensions. An examination of the resulting hydrolyzate in either case reveals that amino acids have been liberated. About twenty-three amino acids have been recognized as the "building blocks" for the majority of proteins, although some forty or more amino acids have been described in the literature as occurring in nature.

From a standpoint of structure an amino acid is an organic acid containing an amino (NH_2) group in the molecule. The amino group is generally located on the carbon adjacent to the acid radical. This is called the α -carbon, and these acids are referred to as the α -amino acids. The length of the acid chain

and the ramifications of that chain vary greatly with different amino acids. Some of them are amino derivatives of the straight-chain acids; others are amino derivatives of a straight-chain acid attached to a ring compound. Some amino acids are derivatives of dicarboxylic acids, whereas others contain two basic amino groups in the molecule. These variations in the structure of the amino acids make it necessary to classify them for convenience of discussion and study.

Classification of amino acids. Amino acids are generally classified according to the structure of the particular acid in question. As a result there are four principal classes of amino acids. They are known as the *monoaminomonocarboxylic acids*, the *monoaminodicarboxylic acids*, the *basic amino acids*, and the *heterocyclic amino acids*. As the name implies, the monoaminomonocarboxylic acids contain one acid group and one amino group. These compounds are neutral to litmus when put into solution. The monoaminomonocarboxylic acids are subdivided into several groups, depending on the nature of the acid ramifications. That is, we find aliphatic acids included under this heading, as well as acids with aromatic nuclei and acids containing sulfur or iodine.

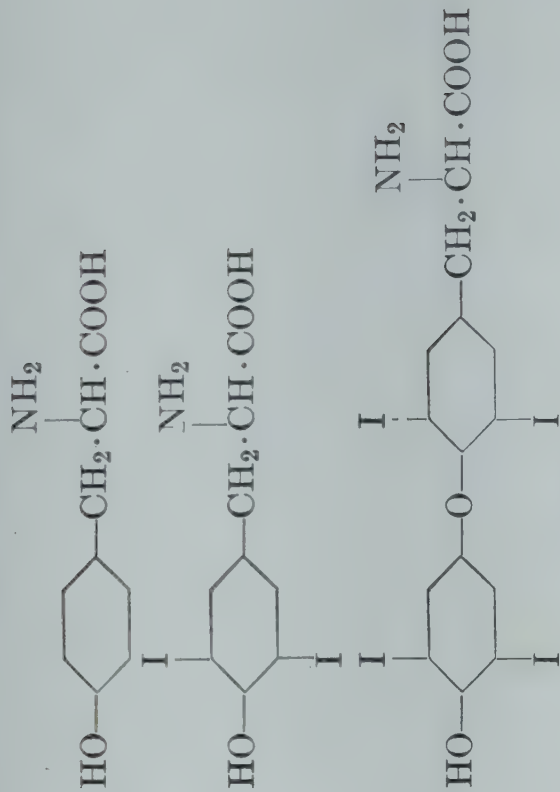
The monoaminodicarboxylic acids are those with one amino group and two acid groups in the molecule. Solutions of these amino acids are acid to litmus. Basic amino acids contain either a second amino group on some carbon other than the α carbon, or they contain some group in the molecule which gives basic-properties to a solution of the amino acid. The fourth group of amino acids contains a heterocyclic nucleus in addition to the amino acid side chain.

Nomenclature of amino acids. With the exception of glycine all amino acids are optically active. This is due to the fact that the carbon to which the amino group is attached is an asymmetric carbon. As in the case of the carbohydrates, this situation gives rise to stereoisomers, depending upon the relative position of the amino group with respect to the carbon axis. If the amino group on the α -carbon atom is on the right side of the axis of symmetry, the amino acid is said to belong to the *D* family, i.e., *D*-alanine. Conversely, if the amino group is on

Common Name	Systematic Name	AMINO ACIDS	Structural Formula
Glycine (glycocol)	Aminoacetic acid		$\begin{array}{c} \text{NH}_2 \\ \\ \text{HCH} \cdot \text{COOH} \end{array}$
Alanine	α -Aminopropionic acid		$\begin{array}{c} \text{NH}_2 \\ \\ \text{CH}_3 \cdot \text{CH} \cdot \text{COOH} \end{array}$
Valine	α -Aminoisovaleric acid		$\begin{array}{c} \text{NH}_2 \\ \\ \text{H}_3\text{C} \quad \text{CH} \cdot \text{CH} \cdot \text{COOH} \\ \\ \text{H}_3\text{C} \end{array}$
Leucine	α -Aminoisocaproic acid		$\begin{array}{c} \text{NH}_2 \\ \\ \text{H}_3\text{C} \quad \text{CH} \cdot \text{CH}_2 \cdot \text{CH} \cdot \text{COOH} \\ \\ \text{H}_3\text{C} \end{array}$
Isoleucine	α -Amino- β -ethyl- β -methyl-propionic acid		$\begin{array}{c} \text{NH}_2 \\ \\ \text{H}_3\text{C} \quad \text{H}_5\text{C}_2 \quad \text{CH} \cdot \text{CH} \cdot \text{COOH} \\ \\ \text{H}_3\text{C} \end{array}$
Serine	α -Amino- β -hydroxypropionic acid		$\begin{array}{c} \text{NH}_2 \\ \\ \text{H}_3\text{C} \quad \text{OH} \quad \text{CH}_2 \cdot \text{CH} \cdot \text{COOH} \\ \\ \text{H}_3\text{C} \end{array}$
Threonine	α -Amino- β -hydroxy- <i>n</i> -butyric acid		$\begin{array}{c} \text{NH}_2 \\ \\ \text{CH}_3 \cdot \text{CH} \cdot \text{CH} \cdot \text{COOH} \\ \\ \text{OH} \end{array}$
Phenylalanine	α -Amino- β -phenylpropionic acid		$\begin{array}{c} \text{NH}_2 \\ \\ \text{CH}_2 \cdot \text{CH} \cdot \text{COOH} \\ \\ \text{C}_6\text{H}_5 \end{array}$

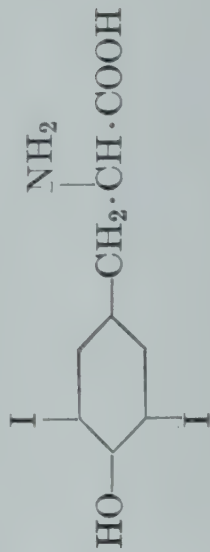
Tyrosine

α -Amino- β -(*p*-hydroxyphenyl)propionic acid



Iodogorgic acid

3,5-Diiodotyrosine



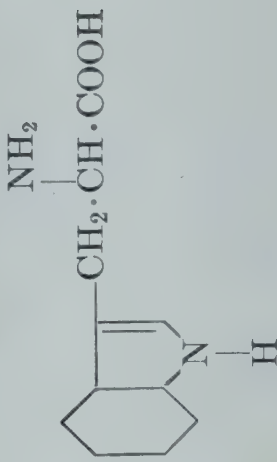
Thyroxine

β -3,5-Diiodo-4-(3',5'-diiodo-4-hydroxy)phenyl- α -aminopropionic acid



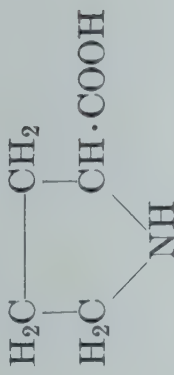
Tryptophan

α -Amino- β -indolpropionic acid



Proline

Pyrrolidine- α -carboxylic acid



Hydroxyproline

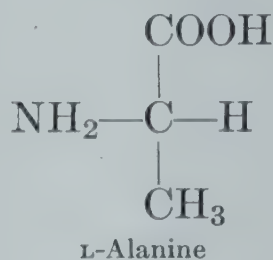
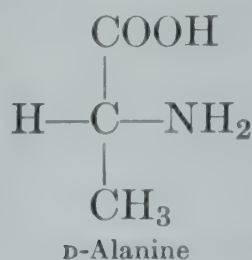
γ -Hydroxypyrrolidine- α -carboxylic acid



AMINO ACIDS (Continued)

Common name	Systematic name	Structural formula
Cystine	Di-(α -amino- β -thiopropionic) acid	$\begin{array}{c} \text{H}_2\text{C}-\text{S}-\text{S}-\text{CH}_2 \\ \qquad \qquad \\ \text{HC}-\text{NH}_2\text{HC}-\text{NH}_2 \\ \qquad \qquad \\ \text{COOH} \qquad \text{COOH} \end{array}$
Methionine	α -Amino- γ -methylthiol- <i>n</i> -butyric acid	$\begin{array}{c} \text{NH}_2 \\ \\ \text{H}_3\text{C}-\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}-\text{COOH} \end{array}$
Aspartic acid	Aminosuccinic acid	$\begin{array}{c} \text{NH}_2 \\ \\ \text{COOH} \cdot \text{CH}_2 \cdot \text{CH} \cdot \text{COOH} \end{array}$
Glutamic acid	α -Aminoglutaric acid	$\begin{array}{c} \text{NH}_2 \\ \\ \text{COOH} \cdot (\text{CH}_2)_2 \cdot \text{CH} \cdot \text{COOH} \end{array}$
Lysine	α - ϵ -Diaminocaproic acid	$\begin{array}{c} \text{NH}_2 \qquad \text{NH}_2 \\ \qquad \qquad \\ \text{CH}_2 \cdot (\text{CH}_2)_3 \cdot \text{CH} \cdot \text{COOH} \end{array}$
Arginine	α -Amino- δ -guanidine- <i>n</i> -valeric acid	$\begin{array}{c} \text{NH}_2 \\ \\ \text{C}=\text{NH} \\ \\ \text{HN}-\text{CH}_2 \cdot (\text{CH}_2)_2 \cdot \text{CH} \cdot \text{COOH} \\ \qquad \qquad \\ \text{N}-\text{CH} \qquad \text{NH}_2 \\ \qquad \qquad \\ \text{HC} \qquad \text{C}=\text{O} \\ \diagup \qquad \diagdown \\ \text{N} \qquad \text{N} \\ \qquad \qquad \\ \text{H} \qquad \text{H} \end{array}$
Histidine	α -Amino- β -imidazolpropionic acid	$\begin{array}{c} \text{NH}_2 \\ \\ \text{CH} \\ \qquad \qquad \\ \text{N} \qquad \text{C}=\text{O} \\ \diagup \qquad \diagdown \\ \text{N} \qquad \text{N} \\ \qquad \qquad \\ \text{H} \qquad \text{H} \end{array}$

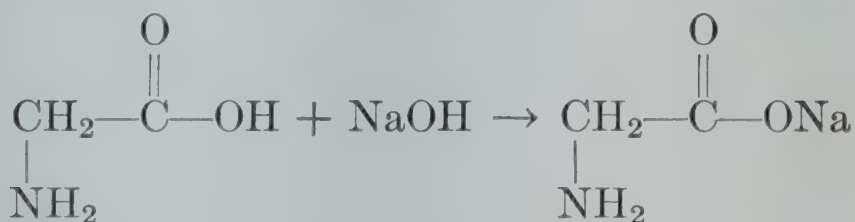
the left side of the axis of symmetry, the compound is an L acid, namely, L-alanine.



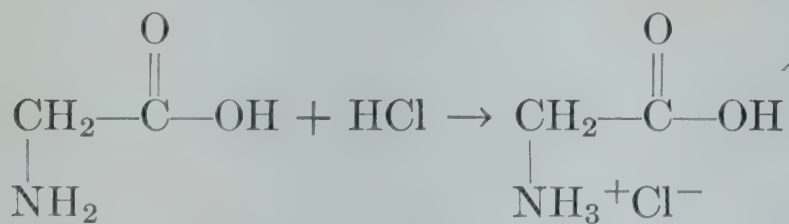
A 50:50 mixture of a D and L form of an amino acid is known as a racemic mixture and is optically inactive, owing to external compensation. Such a compound would be designated as a DL compound, namely, DL-alanine.

Reactions of amino acids. Amino acids will enter into a large number of different reactions, owing to the presence of acid and basic groups. A few of these reactions will be described below:

1. *Amino acids will react with bases to form salts.*

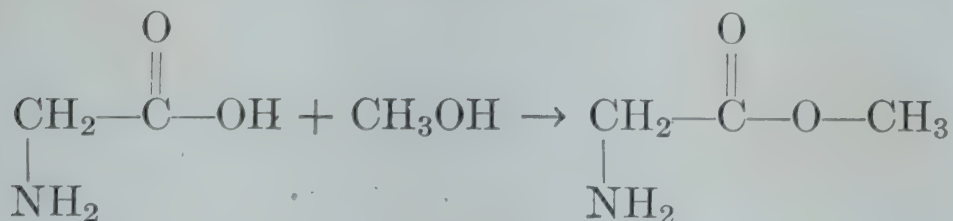


2. *Amino acids will react with acids to form salts.*

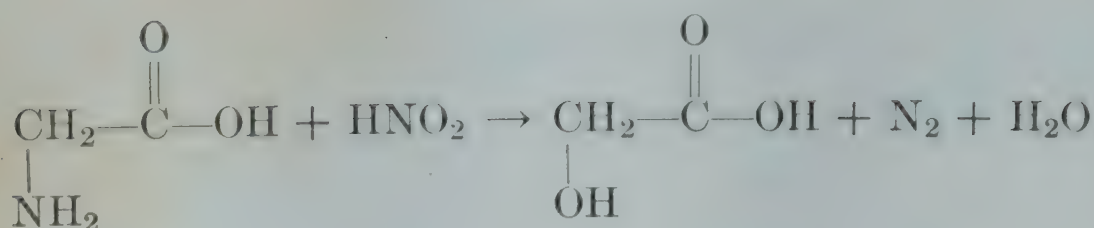


3. *Amino acids can be methylated.*

4. *When refluxed with an alcohol, esters are formed.*

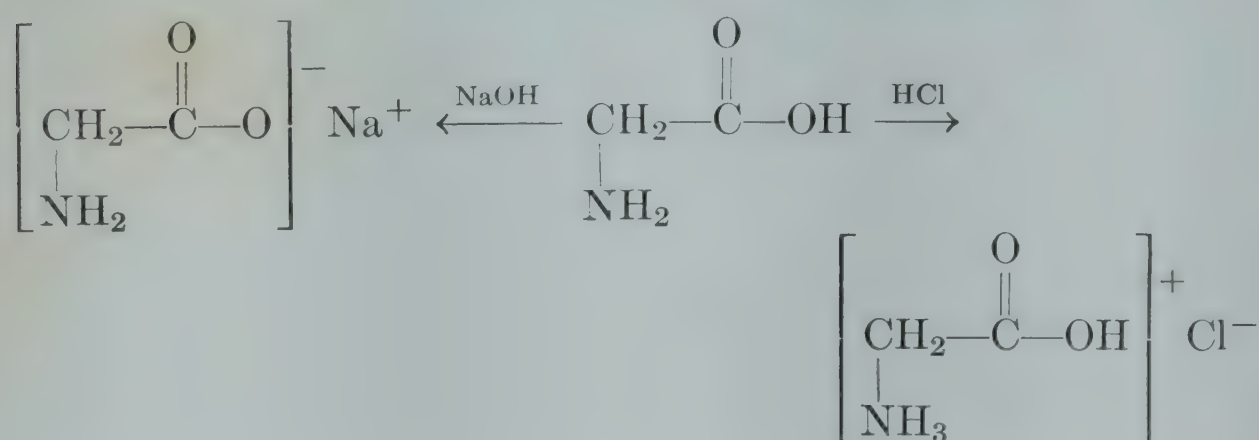


5. *Amino groups are decomposed when the acid is treated with nitrous acid.*

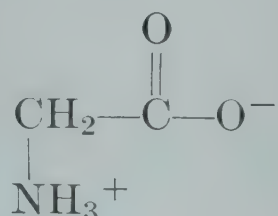


Since proteins contain free amino and free carboxyl groups, one can expect that they will enter into the same reactions as the amino acids. It has been shown that this is the case, since salts of proteins, methylated proteins, esters of proteins, and nitrous acid derivatives of proteins have been formed and isolated.

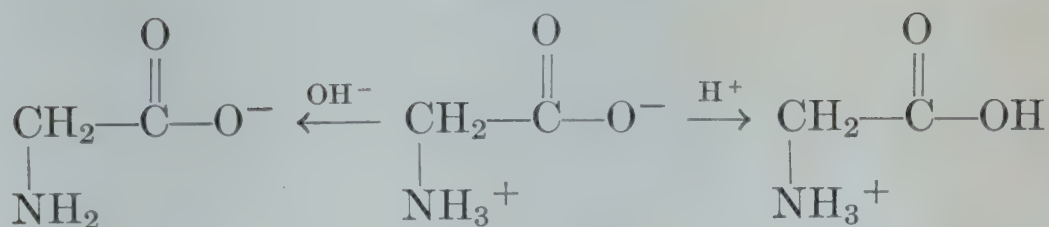
Zwitter ions. As has been stated previously, amino acids (or proteins) will react with acids or bases to form salts. These reactions can be summarized by the following equation:



These equations imply that the addition of an acid results in the ionization of the amino group, and the addition of a base causes the ionization of the carboxyl group. If such were the case, a protein or an amino acid in water solution would not be ionized. Recent evidence seems to indicate that the amino acid or protein exists in a highly dissociated form called a *zwitter ion* or a *dipolar ion*. Such an ion may be represented as follows:



Assuming that amino acids in water solution do exist in dissociated form, the equations representing the reaction of the amino acid with a base and an acid may be represented as follows:

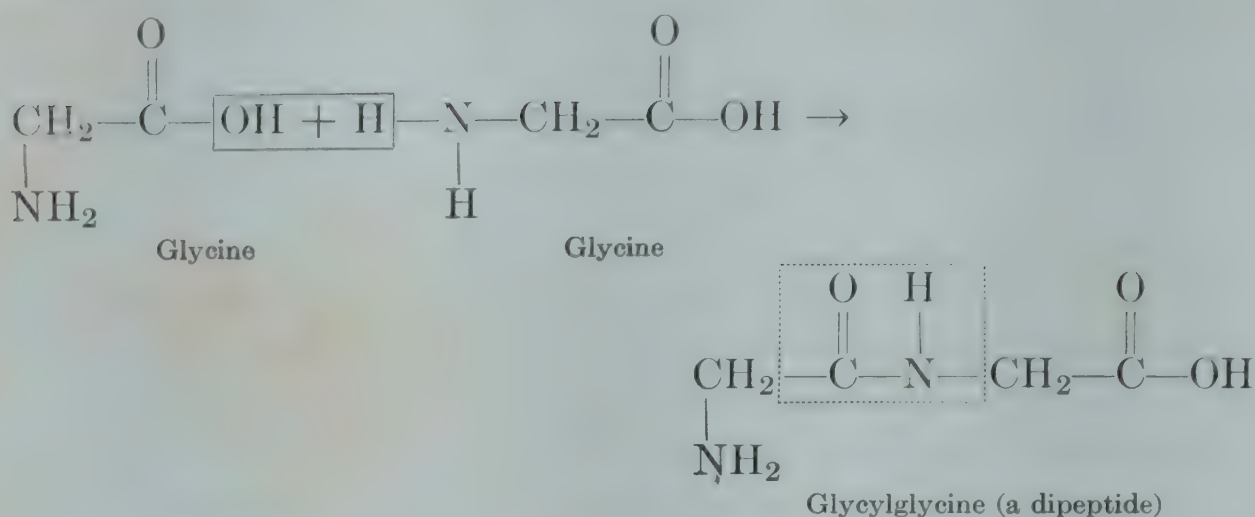


Since in the dipolar theory the amino acid is completely dissociated, the addition of an acid or a base merely tends to depress the ionization of the carboxyl and the amino groups, respectively.

Isoelectric point. All amino acids (and proteins) are amphoteric; that is, they will dissociate both as weak acids and weak bases. Their dissociation as acids or bases when in solution depends on the *pH* of the solution. When the hydrogen ion concentration of the solution is lower than a certain critical value, the amino acid will dissociate as an acid. Conversely, when the hydrogen ion concentration is above this critical value, the amino acid will act as a base. At the critical *pH* mentioned in the foregoing statements, the amino acid, although actually dissociated, is neutralized internally and exhibits equal acid and basic properties. This *pH* is called the *isoelectric point* of the amino acid (or protein) in question.

That each amino acid or protein has its own isoelectric point may be demonstrated in another way. It has been shown that, when a constant current is passed through a solution of an amino acid, the acid will migrate in one direction or in the other, depending on whether an acid or an alkali is added to the solution. It seems obvious, therefore, that at a certain *pH* the amino acid will not migrate in either direction. This *pH* is the isoelectric point of the amino acid or protein.

Peptide formation. Each amino acid has at least one carboxyl group and one amino group. These groups are responsible for the acidic and basic properties of the acids. (It has been shown that amino acids can enter into a number of different reactions with various chemical reagents. They can also react among themselves to form long-chain, high molecular weight compounds, called *polypeptides*.) When two amino acids react, they are joined together through the amino group of one acid and the carboxyl group of a second acid with the evolution of one molecule of water. The reaction may be illustrated thus:



The linkage between the carboxyl group of the first molecule of glycine and the amino group of the second glycine is called the *peptide linkage*. The peptide linkage is the characteristic linkage found in all proteins. When two amino acids are joined together through the peptide linkage, the resulting compound is called a *dipeptide*. Dipeptides are named according to the amino acids included in their structure. Thus it can be seen that the name for the dipeptide shown above is glycylglycine. If one molecule of glycine is combined with one molecule of alanine, the name of the resulting dipeptide would be glycylalanine.

When three amino acids are joined together in this fashion, a tripeptide is formed. It will be noted that a tripeptide contains three amino acids but only two peptide linkages. The name of a characteristic tripeptide is glycylalanyls erine.

When x amino acids are joined together through the peptide linkage, a polypeptide is formed. A polypeptide contains x amino acids and $x - 1$ peptide linkages. Fischer, a very famous protein chemist, was able to synthesize a polypeptide containing 18 amino acids. This polypeptide had a molecular weight of 1213 and contained 15 glycine and 3 leucine radicals. Although this synthesis has been considered a great feat from the standpoint of laboratory technique, the resulting compound has not even approached the simplest proteins in complexity. A very simple protein, egg albumin, contains about 300 acid radicals, whereas some of the more complex proteins contain well over 1000 acid fragments.

Molecular weight of proteins. Since proteins are such complex materials, it is extremely difficult to determine their molecular

weight. The usual techniques for the determination of molecular weight, i.e., osmotic pressure measurements or freezing-point measurements, are not sufficiently accurate when used on proteins. The ultracentrifuge method developed by Svedberg has proved the most satisfactory method for the determination of the molecular weight of proteins. This method is based on the fact that the sedimentation rate (rate of settling) of a protein is proportional to its molecular weight. The ultracentrifuge, an improved oil turbine, operates at speeds varying from 5000 to 80,000 revolutions per minute. At the latter speed the centrifugal force exerted on a sample is greater than 400,000 times the force of gravity. By the use of the ultracentrifuge, Svedberg and co-workers have shown that proteins vary in molecular weight from 15,000 to more than 10,000,000.

Structure of proteins. In spite of the recent advances in the field of chemistry, little is known concerning the structure of proteins. If we are to assume that Fischer's work on the peptide linkage is correct, a protein must be composed of a great number of amino acid fragments joined together through the peptide linkage. The exact number and order of the amino acids is unknown, but it is known that, if the number and order are changed, a different protein is produced. Theoretically, then, there is an infinite number of possibilities for the production of proteins from the available number of amino acids. There are several different theories dealing with how the protein molecule is shaped. Some authorities believe that the long chain of amino acids is bent back on itself in loop fashion, forming a rather compact molecule. Some contend that the amino acid chain is bent into a series of hexagons, whereas others say that the protein molecule is composed of a number of peptide chains lying parallel to each other. It is apparent from these differences in opinion that the nature of protein structure is still uncertain and that a lot of research is required before the problem can be settled definitely.

Color reactions of proteins. Many color tests for proteins have been devised. Generally speaking, these color tests depend on the presence of some particular amino acid in the protein molecule, or on a peculiar linkage that might be present in some protein molecules.

Biuret reaction. When a protein is mixed with a solution of sodium hydroxide and a weak solution of copper sulfate, a violet color is produced. This is a test for the peptide linkage and will be positive when two or more peptide linkages are present. The violet color is due to the formation of linkages similar to those present in *biuret*, having the formula



Xanthoproteic reaction. When a protein is heated in the presence of nitric acid, a yellow color is produced which changes to an orange color upon the addition of alkali. Any amino acid containing a benzene ring will give this test. Tyrosine and tryptophan are among the amino acids that give the test.

Millon's reaction. When a protein is heated with Millon's reagent (solution of mercuric nitrite and nitrate in nitrous and nitric acid), a red precipitate is produced. A positive test is due to the presence of a phenolic group in the protein molecule. Tyrosine is an example of an amino acid that contains this structure.

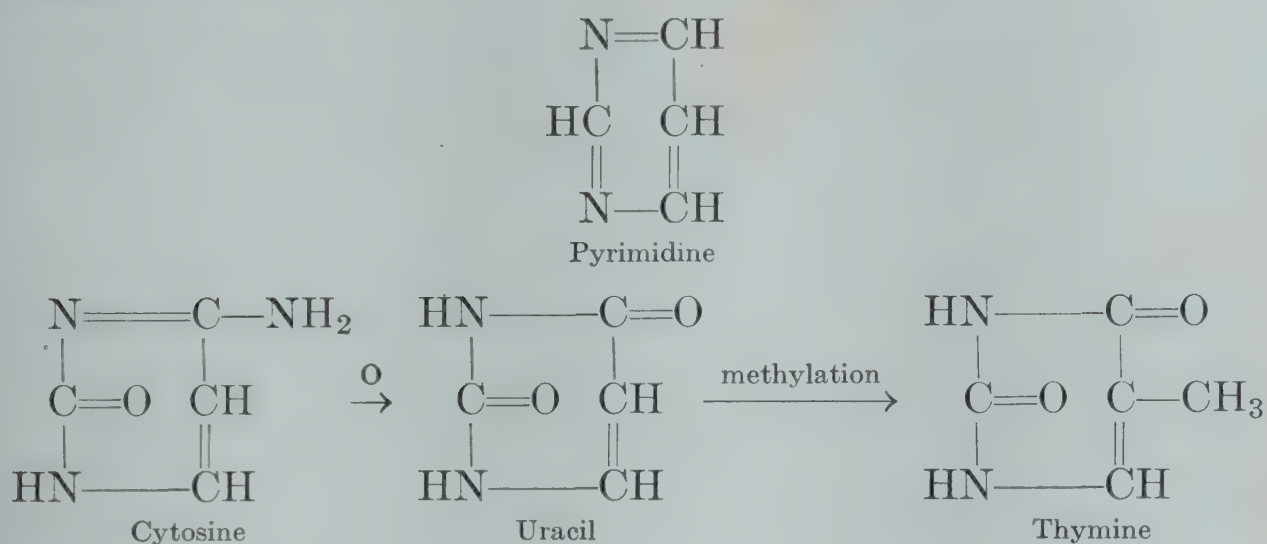
NUCLEOPROTEINS

Occurrence and properties. As the name implies, nucleoproteins are found in all nucleated cells of plants and animals. Although it was originally thought that nucleoproteins exist only in the nucleus, recent studies indicate that some of these compounds are present in the cytoplasm of the cell.

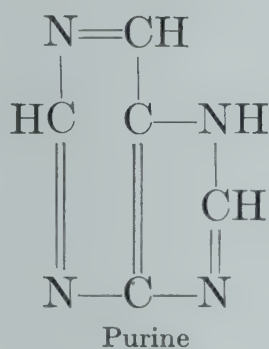
All nucleoproteins have certain characteristics in common. They are acidic in reaction and are soluble in dilute solutions of alkali. On hydrolysis, nucleoproteins are split into two principal parts, namely, a *protein* and a *nucleic acid*. The proteins that are generally present in these compounds are simple proteins such as albumins, histones, and protamines. On hydrolysis the nucleic acid fraction is found to yield phosphoric acid, D-ribose, and derivatives of the organic bases known as the *pyrimidines* and *purines*.

Pyrimidines. Pyrimidine is the parent substance for a number of compounds found in nucleic acid. Following is the structure

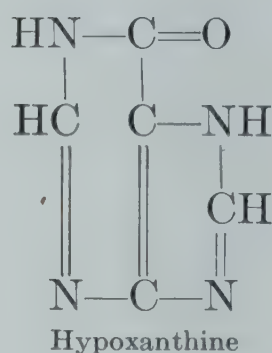
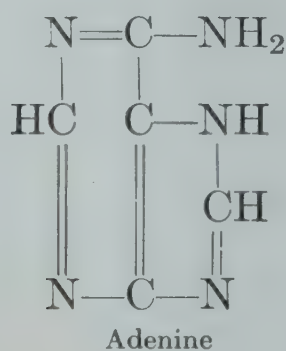
for *pyrimidine*, *cytosine*, *uracil*, and *thymine*, some of the important derivatives which have been isolated from various nucleic acids.

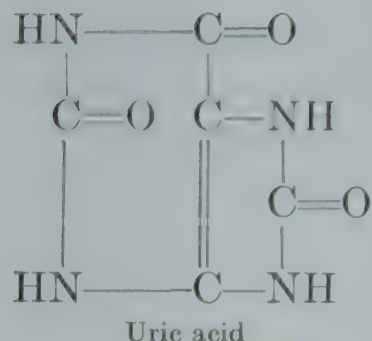
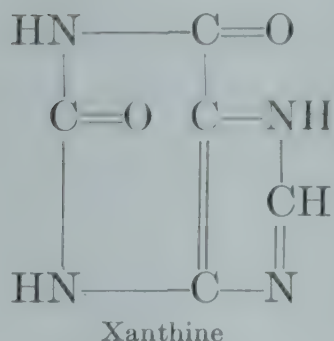
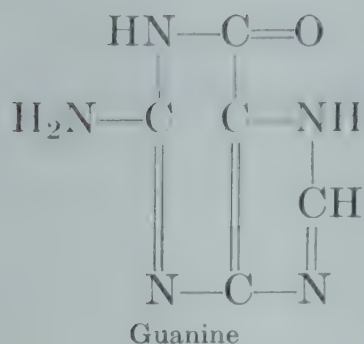


Purines. The parent substance of the purine bases is the organic compound, *purine*, which has been assigned the following structure:

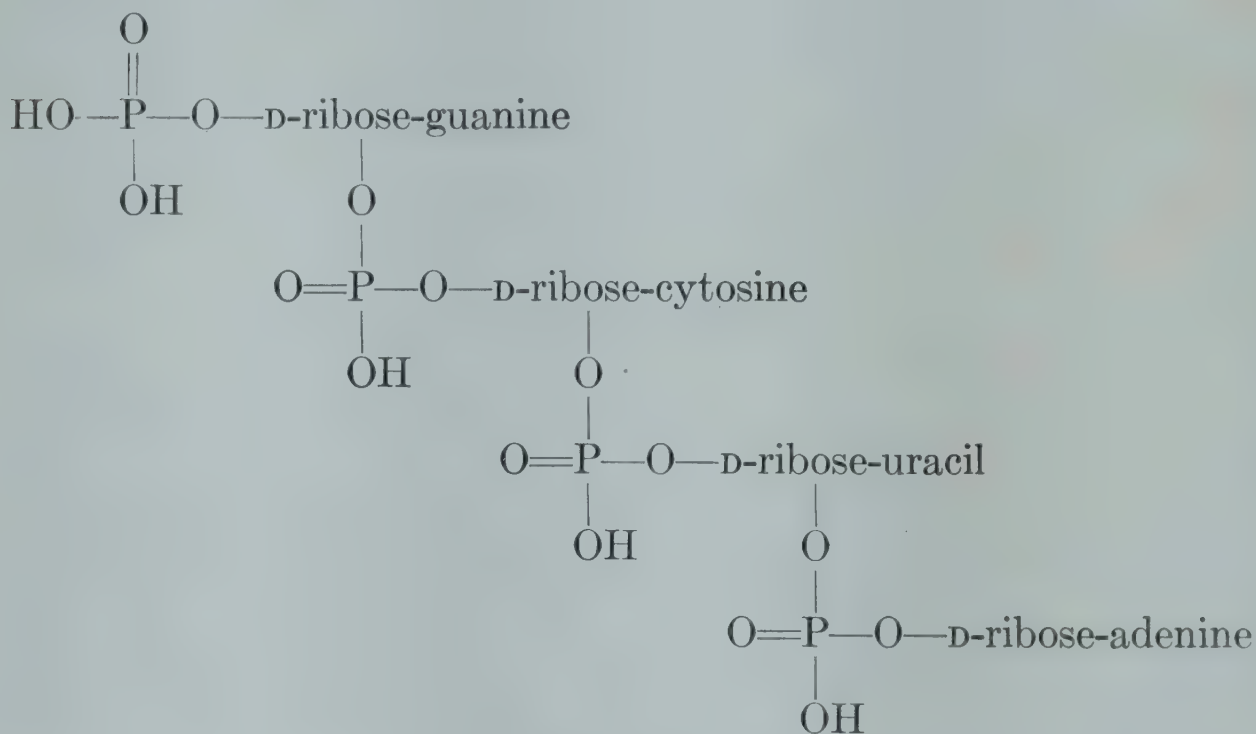


Adenine and *guanine*, derivatives of purine, are found in a great number of the nucleic acids studied thus far. Closely related to these bases are the compounds *hypoxanthine* and *xanthine*. Hypoxanthine on oxidation yields xanthine which in turn yields *uric acid* on oxidation. Following are the structures for these compounds:





Nucleic acid. Jones and Levene hydrolyzed the nucleic acid isolated from yeast and found that it could be split into four components, each of which was called a *nucleotide*. Upon hydrolysis a nucleotide yields phosphoric acid, D-ribose, and a purine or pyrimidine base. Nucleic acid is, therefore, a tetranucleotide. Following is the proposed structure for a typical nucleic acid molecule:



A typical nucleic acid

When a mononucleotide is hydrolyzed so that the phosphoric acid molecule is removed, a compound known as a *nucleoside* is formed. Upon hydrolysis a nucleoside yields ribose and a purine or pyrimidine base.

7 · Enzymes

One of the characteristics of normal living cells is that the reactions which are constantly taking place within them are controlled and regulated. The rate of hydrolysis and esterification, oxidation and reduction, degradation and synthesis, and other reactions within the cells can evidently be changed as the demands of the living organism change. Food eaten by the animal undergoes a series of hydrolytic reactions in the mouth, the stomach, and the intestines. These reactions outside the cells are also controlled. The group of agents which control these reactions through their catalytic effect are called *enzymes*.

Definition of enzymes. *Enzymes are protein catalysts which regulate the rates of specific reactions both within and without the living cells that produced them.*

Thus far all the enzymes that have been separated, purified, and crystallized have been found to be composed of proteins, although they often contain other substances which are essential to their actions. Such substances as the vitamins riboflavin, niacin, thiamine, and pyridoxal, and the elements copper, iron, and zinc are essential parts of certain enzymes. These substances are known to be needed for the normal growth and well-being of living organisms and are needed because they form parts of some enzymes. Green has postulated that any vitamin or element which is required in very small amounts for the well-being of living things will be found to be an essential part of some enzyme system. So far, no information has been found that refutes this theory.

Since enzymes are of a protein nature, any agents such as heat, acids, or alkalies that will denature a protein will also destroy the activity of an enzyme. Thus one of the important characteristics of enzymes is the fact that, when heated, their

activities are readily lost. A temperature of 80° C for a few minutes is sufficient to inactivate most enzymes. If the enzyme preparation is dry, the enzymes are more resistant to high temperatures.

Most enzymes are soluble in water. A few, such as castor bean lipase, have such low solubility that they are usually called insoluble. Enzymes are also soluble in dilute acetone, alcohol, and glycerol but are not soluble in the fat solvents. Enzymes found in nature may be bound to or surrounded by other proteins. To make a solution of such enzymes it is necessary to remove the surrounding material by digesting or dissolving these interfering compounds.

The determination of the molecular weights of enzymes presents the same difficulties as the determination of the molecular weights of proteins. Determinations made by existing methods show that the approximate molecular weights of some enzymes are as follows:

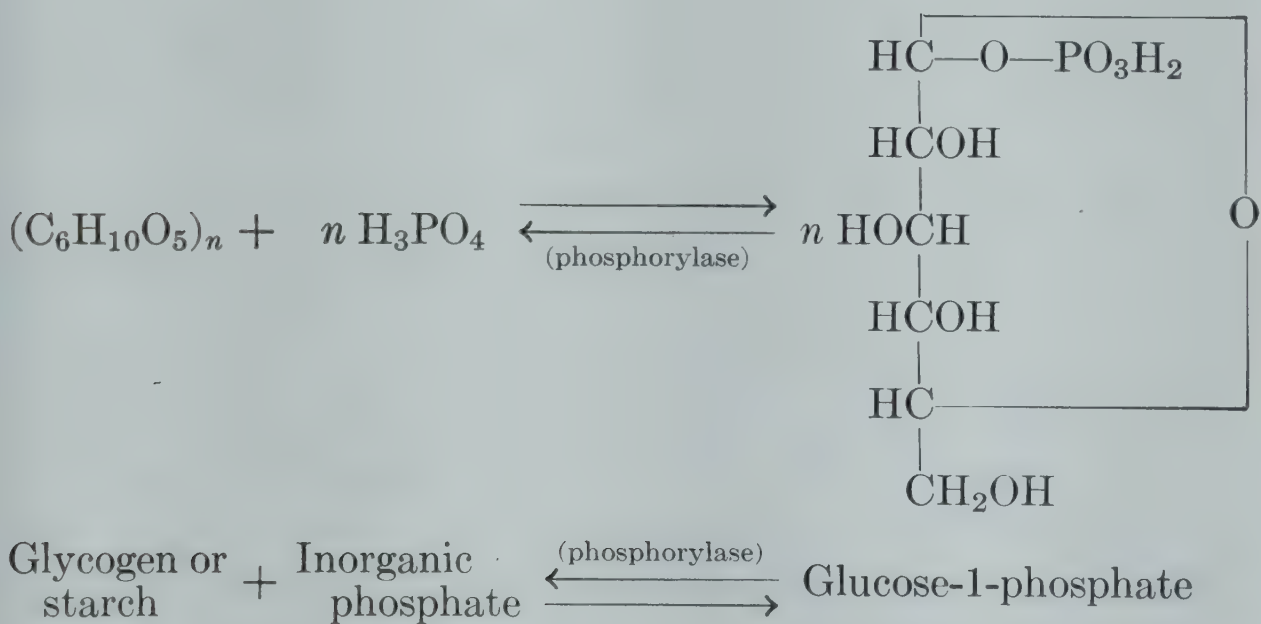
Invertase	20,000
Pepsin	36,000
Enolase	63,000
Warburg and Christian's yellow enzyme	80,000
Catalase	225,000
Urease	483,000

Because of the large molecular size of these protein substances, it is to be expected that enzymes will form colloidal solutions or suspensions. These enzymes in solution do not pass through the common semipermeable membranes such as cellophane and sausage skins. We can readily see how important it is to have most biological catalysts of such large size that they are confined to the cells that produce them. This allows cells to be "specialists" and carry out the specific reactions required of various organs in both plants and animals.

Enzymes as catalysts. Our definition of enzymes states that they are catalysts. A familiar example of the action of a catalyst is the reaction which most students perform in general inorganic chemistry, in which oxygen is produced by heating potassium chlorate. But oxygen is not liberated readily until a small amount of manganese dioxide (MnO_2) is added to the

KClO_3 , whereupon oxygen is given off copiously. In this case the manganese dioxide acts as a catalyst and accelerates the decomposition of the chlorate. It is characteristic that only small amounts of the catalytic substance are needed to accelerate the reaction manyfold. Enzymes also function in very small amounts and yet accelerate a chemical reaction tremendously. Under favorable conditions they can repeat their action almost indefinitely.

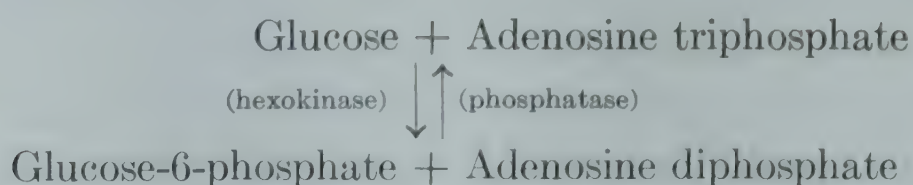
Not only do enzymes increase the rate of biochemical reactions, but in many cases they apparently start the reaction, as seems to be true for many other catalysts. In any reaction accelerated by an enzyme, the final equilibrium attained is independent of the amount of enzyme present. Since the final equilibrium of the catalyzed reaction is not changed, the effect of adding increasing amounts of an enzyme is to shorten the time needed to attain this equilibrium point. If such reactions are reversible, the same enzyme may catalyze the reaction in both directions. An example of such a reversible reaction is the following:



At 25°C and $p\text{H}$ 7.0, equilibrium is established with 77 per cent inorganic phosphate and 23 per cent glucose-1-phosphate. The same equilibrium is achieved with the enzyme phosphorylase, whether the reaction proceeds from the left or from the right.

However, for some apparently reversible reactions one enzyme is required for the change to take place in one direction and

another is required for a reversal of the reaction. An illustration of such a reaction is:



Another well-known biochemical reaction which proceeds only in one direction is the breakdown of hydrogen peroxide to oxygen and water under the influence of catalase.

Extracellular and intracellular enzymes. Although enzymes are synthesized by living cells, many of these catalysts are secreted by and function outside the cells that produced them. Enzymes secreted by cells are called *exoenzymes* or *extracellular enzymes*. Extracellular enzymes are found in the mouth, the stomach, and the intestines of animals and in the “pitcher” of insectivorous plants.

Endoenzymes or *intracellular enzymes* operate within the cells in which they are produced. Most enzymes in plants and animals belong to this group. Those enzymes which are bound to protoplasm and must be liberated by proteolytic action are called *desmoenzymes*. Soluble intracellular enzymes are called *lyoenzymes*.

Occurrence and distribution. Enzymes occur in all living cells, but not all enzymes are found in all cells. Hundreds of different kinds of enzymes are known to exist. These catalyze a wide variety of biochemical reactions, many of which are localized in certain organs or are peculiar to certain species of plant or animal life. Thus pepsin is produced only in the cells of the gastric mucosa, and trypsin only in the pancreas. In the plant world lipases are not generally distributed but are found chiefly in plants that produce oil-bearing seeds.

Some enzymes are present in most forms of life. Catalase, which breaks down hydrogen peroxide to molecular oxygen, occurs in all higher forms of plant and animal life. It is absent only in certain species of microorganisms such as hot-spring algae. Cytochrome oxidase also seems to be present in most forms of life. The latter plays an important role in respiration

since it functions as a catalyst for the reaction in which hydrogen combines with molecular oxygen to form water.

Sumner and Somers have estimated that a single cell may contain a thousand different enzymes. This is easily possible in both liver and yeast cells in which the number of known reactions probably approaches a thousand in each case.

Nomenclature. Systematic names for enzymes are formed by adding the suffix -ase to (1) the name of the substance acted upon (the *substrate*) or (2) the nature of the catalyzed reaction. Thus (1) the enzyme sucrase acts on sucrose, a proteinase (or protease) acts on a protein, urease on urea, and pectase on pectin. (2) Oxidases catalyze oxidations, dehydrogenases split off hydrogen, and mutases cause molecular rearrangements. The source of the enzyme is sometimes used as a part of its name, since enzymes from different sources often have different characteristics. Salivary amylase, liver catalase, and ricinus lipase are examples of such names. Even this is not enough to differentiate some kinds of enzymes. Cow-liver catalase differs from horse-liver catalase since it is much less soluble and shows other characteristic differences. Several non-systematic names are in general use. Pepsin, trypsin, rennin, papain, bromelin, and ficin are older names still used for various proteinases.

Although most enzymes are simple proteins, some of them consist of a protein combined or associated with a non-protein component, as was indicated in a previous paragraph. The complete intact enzyme is called the *enzyme system* or *holoenzyme*, the protein part of the holoenzyme which determines its specificity is called the *apoenzyme*, and a non-protein compound needed for its action is called the *coenzyme*. The coenzyme can be removed from the holoenzyme by dialysis, or by other methods. Neither the remaining apoenzyme nor the separated coenzyme are active by themselves, but if the two materials are brought together their activity as an enzyme is restored. Several coenzymes of known chemical structure have been separated and described. They are heat-stable, crystalloid compounds. Metallic ions which may form a part of some enzymes or are required for the action of others are not called coenzymes. Some holoenzymes with their coenzymes are listed as follows:

HOLOENZYMES	COENZYMES
Carboxylase	Coccarboxylase (thiamine pyrophosphate)
Malic acid dehydrogenase (and other dehydrogenases)	Coenzyme 1 (diphosphopyridine nucleotide containing niacin)
Glutamic acid dehydrogenase (and other dehydrogenases)	Coenzyme 2 (triphosphopyridine nucleotide containing niacin)
Warburg and Christian's yellow enzyme	Riboflavin phosphate
Amino acid decarboxylase	Pyridoxal phosphate

Specificity. Every enzyme catalyzes a specific reaction or type of reaction. Some enzymes are able to catalyze only a single reaction of one substrate. Such an enzyme is urease, which catalyzes the decomposition of urea into ammonia and carbon dioxide. No other compound is affected. Other enzymes are active with several substrates but are specific for a particular stereoisomeric structure which each of the substrates must contain. Thus α -glucosidase acts on α -glucosides such as α -methyl-D-glucoside and maltose, but does not act on β -methyl-D-glucoside or cellobiose. Many enzymes catalyze the hydrolysis of a large group of similar substances. Pepsin acts on all soluble

native proteins. It is evident that the specificity of enzymes is a relative matter.

It is difficult to find a satisfactory explanation for the specificity of enzymes. Emil Fischer suggested that an enzyme to be effective must fit the molecule of the substrate somewhat as a key fits a lock. This can be interpreted as allowing the enzyme a rela-

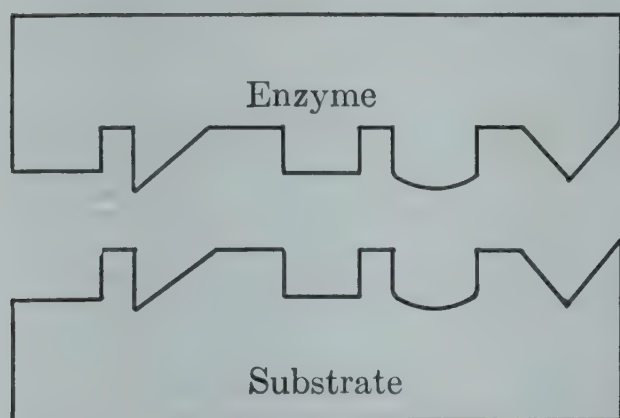


FIG. 5. Enzyme substrate relationship.

tively close approach to the substrate. Some enzymes seem to be "master keys," for they can fit into several substrates possessing certain similar atomic groupings in their molecules. This theory holds that the specificity of enzymes is determined primarily by the size and shape of the substrate molecules rather than by their chemical properties.

The lock and key theory is not a unique theory coined to explain the specificity of enzyme action. This theory was first suggested by Paul Ehrlich to explain the specificity of antibodies.

Classification. An ideal classification of enzymes would group enzymes of similar structure and composition in a single class. Wherever possible this has been done in the classification used in this textbook. For example, the enzymes requiring pyridoxal phosphate are grouped together. In general, however, the classification on pp. 118–125 is based on the nature of the catalyzed reaction and the type of substrate.

Autolysis. After cells die, a process of decomposition takes place which results in the hydrolysis of most of the proteins. This destruction, which is brought about by agents present in the cells, will take place even in the presence of an antiseptic which prevents the growth of microorganisms. This self-digestion of dead tissue, *autolysis*, is due to proteinases called *cathepsins*, to lipases, and to other enzymes.

Preparation and crystallization. Enzymes are prepared by the same general methods used for the separation of proteins. When such preparations are sufficiently purified, it is possible to crystallize some of them. This is the ultimate aim of enzymologists in purifying enzymes, because a crystallized product approaches a chemically pure compound. J. B. Sumner made the first crystalline enzyme preparation in 1926. Twenty years later he was awarded a Nobel prize for this outstanding work. About thirty other enzymes have been prepared in crystalline form, since Sumner crystallized urease from a jack bean extract.

As a rule, water is the best solvent for enzymes, but it is sometimes advantageous to use dilute acetone, glycerol, or ethyl alcohol to prevent solution of other substances. Animal glands, such as liver, and plant materials, such as seeds, should be ground and extracted with acetone or ether to remove lipids before extraction with water. The water extract containing the enzyme is separated from the residue by centrifuging or filtering, and the enzyme may be precipitated by half-saturating the solution with ammonium sulfate. Further purification and crystallization depends on the particular enzyme preparation. The isolation of urease was accomplished only after many years of pre-

CLASSIFICATION OF ENZYMES

HYDROLYTIC ENZYMES

Name	Substrate	End Products
I. Esterases	Esters	Acids + alcohols
1. Lipases	Fats	Fatty acids + glycerol
a. Ricinus lipase	Fats	Fatty acids + glycerol
b. Pancreatic lipase	Choline esters	Choline + acid
2. Choline esterase	Cholesterol esters	Cholesterol + fatty acids
3. Cholesterase	Chlorophyll	Chlorophyllide + phytol
4. Chlorophyllase	Lecithin	Lysolecithin + fatty acid
5. Lecithinase A	Lysolecithin and lecithin	Glycerophosphate + fatty acid
6. Lecithinase B		
7. Phosphatases		
a. Monophosphatases	Nucleotides	Nucleosides + H_3PO_4
(1) Nucleotidase	Glycerophosphate	Glycerol + H_3PO_4
(2) Glycerophosphatase	Phosphorylcholine	Choline + H_3PO_4
(3) Choline phosphatase	Phytates	Inositol + H_3PO_4
(4) Phytase	Fructose-1,6-diphosphate	Fructose-6-phosphate + H_3PO_4
b. Hexose diphosphatase	Adenosine triphosphate	Adenosine diphosphate + H_3PO_4
c. Adenosine triphosphatase	ATP + glucose or fructose	ADP + hexose-6-phosphate
d. Hexokinase (synthesizing)	Pectin	Pectic acid + MeOH
8. Pectase	Tannin	Glucose + gallic acid
9. Tannase		
10. Polynucleotidases		
a. Ribonuclease	Yeast nucleic acid	Nucleotides
b. Thymonucleodepolymerase	Thymonucleic acids	Nucleotides

II. Carbohydrases

A. Simple glycosidases and saccharidases

1. β -Fructofuranosidase (Invertase) (Sucrase)	Sucrose Raffinose Gentianose Stachyose	Fructose + glucose Fructose + melibiose Fructose + gentiobiose Fructose + trisaccharide
2. α -Galactosidases a. Melibiase	Melibiose	Glucose + galactose
3. β -Galactosidases a. Lactase	Lactose	Glucose + galactose
4. α -Glucosidases a. Maltase b. Trehalase c. Melezitase	Maltose Trehalose Melezitose	Glucose Glucose Glucose + fructose
5. β -Glucosidases (emulsin) a. Cellobiase b. Gentiobiase c. Amygdalase d. Prunase e. Salicinase	Cellobiose Gentiobiose Amygdalin Prunasin Salicin	Glucose Glucose Glucose + prunasin Glucose + <i>d</i> -mandelonitrile Glucose + saligenin
6. Thioglucosidases a. Sinigrase (+ a sulfatase)	Sinigrin	Glucose + allylthiocyanate + KHSO ₄
7. Nucleosidases a. Purine nucleosidase b. Pyrimidine nucleosidase	Purine nucleosides Pyrimidine nucleosides	Pentose + purine bases Pentose + pyrimidine bases

CLASSIFICATION OF ENZYMES (Continued)

HYDROLYTIC ENZYMES (Continued)

Name	Substrate	End Products
B. Polysaccharidases		
1. Amylases		
a. α -Amylase (liquefying)	Starch	Dextrins
b. β -Amylase (saccharifying)	Starch	Maltose
2. Inulase	Inulin	Fructose
3. Cellulase	Cellulose	Cellobiose
4. Lichenase	Lichenin	Cellobiose
5. Cytases		
a. Hexosanases	Hexosans	Simple sugars
b. Pentosanases	Pentosans	Simple sugars
6. Chitinase	Chitin	N-Acetylglucosamine
7. Protopectinase	Native pectins	Soluble pectins
8. Pectinase (pectolase)	Pectic acid and soluble pectins	Galactose + galacturonic acid
III. Proteinases		
1. Pepsin	Native proteins	Proteoses and peptones
2. Trypsin	Native proteins, proteoses, peptones	Polypeptides and amino acids
3. Chymotrypsin	Native proteins, proteoses, peptones	Polypeptides and amino acids
4. Papain	Native proteins	Polypeptides and amino acids
5. Bromelin	Native proteins	Polypeptides and amino acids
6. Ficin	Native proteins	Polypeptides
7. Cathepsin	Native proteins	Proteoses and peptones

8. Rennin	Casain	Paracasein
9. Keratinase	Keratin	Amino acids
IV. Peptidases		
1. Polypeptidases	Polypeptides	Peptides and amino acids
a. Aminopolypeptidases	Polypeptides with free amino groups	Peptides and amino acids
b. Carboxypeptidases	Polypeptides with free carboxyl groups	Peptides and amino acids
2. Dipeptidase	Dipeptides	Amino acids
3. Prolylpeptidases	Proline peptides	Proline + peptides
a. Prolinase	Proline peptides with proline CO—NH peptide linkage	Proline + peptides
b. Prolidase	Proline peptides with proline N—CO peptide linkage	Proline + peptides
4. Hippuricase (histozyme)	Hippuric acid	Benzoic acid + glycine
V. Amidases		
1. Allantoinase	Allantoin	Glyoxylic acid + urea
2. Asparaginase	Asparagine	Aspartic acid + NH_3
3. Glutaminase	Glutamine	Glutamic acid + NH_3
4. Histidase	Histidine	Glutamic acid + formic acid + NH_3
5. Urease	Urea	$\text{CO}_2 + \text{NH}_3$
6. Benzamidase (synthesizing)	Benzoic acid + NH_3	Benzamide
VI. Aminases		
A. Nuclein desaminases		
1. Adenase	Adenine	Hypoxanthine + NH_3
2. Adenosine desaminase	Adenosine	Hypoxanthosine + NH_3
3. Adenylic acid desaminase	Adenylic acid	Inosine-5-phosphoric acid + NH_3

CLASSIFICATION OF ENZYMES (Continued)

HYDROLYTIC ENZYMES (Continued)

Name	Substrate	End Products
4. Cytidine desaminase	Cytidine	Uridine + NH_3
5. Guanase	Guanine	Xanthine + NH_3
6. Guanosine desaminase	Guanosine	Xanthosine + NH_3
7. Guanylic acid desaminase	Guanylic acid	Xanthylic acid + NH_3
B. Other aminases		
1. Arginase	Arginine	Ornithine + urea
2. Aspartase	Aspartic acid	Fumaric acid + NH_3
3. Canavanase	Canavanine	Canaline + urea
OXIDIZING ENZYMES		
I. Oxidases		
A. Iron oxidases		
1. Catalase	Hydrogen peroxide	Water + O_2
2. Peroxidase	H_2O_2 + aromatic substrate	H_2O + oxidized substrate
3. Cytochrome oxidase	Reduced cytochrome C	Oxidized cytochrome C
4. Cytochrome peroxidase	H_2O_2 + reduced cytochrome C	H_2O + oxidized cytochrome C
B. Copper oxidases		
1. Tyrosinase (monophenol oxidase)	Tyrosine	Dihydroxyphenylalanine
	Catechol	<i>o</i> -Quinone
	<i>p</i> -Cresol	Homocatechol
	Phenol	Catechol
	Hydroquinone	Quinone
2. Laccase (polyphenol oxidase)	Pyrogallol	Purpurogallin
	Ascorbic acid	Dehydroascorbic acid
3. Ascorbic acid oxidase		

II. Dehydrogenases

A. Flavoproteins (containing riboflavin)

1. Yellow enzyme of Warburg and Christian	Reduced coenzyme 2	Oxidized coenzyme 2
2. Diaphorase (Straub flavoprotein)	Reduced coenzyme 1 or 2	Oxidized coenzyme
3. Xanthine oxidase (Scharidinger enzyme)	Hydrated xanthine	Uric acid
4. D-Amino acid oxidase	D-Amino acid	α -Keto acid + NH_3 + H_2O_2
5. L-Amino acid oxidase	L-Amino acid	α -Keto acid + NH_3 + H_2O_2
B. Dehydrogenases containing coenzyme 1 or 2 (compounds of nicotinamide)		
1. Glucose dehydrogenase	Glucose	Gluconic acid
2. Hexose-6-phosphate dehydrogenase	Hexose-6-phosphate	Phosphohexonic acid
3. α -Glycerophosphate dehydrogenase No. 1	α -Glycerophosphate	3-Phosphoglyceric aldehyde
4. Phosphoglyceric aldehyde dehydrogenase	3-Phosphoglyceric aldehyde + phosphate	1,3-Disphosphoglyceric acid
5. Lactic dehydrogenase (animal)	Lactic acid	Pyruvic acid
6. Isocitric dehydrogenase	Isocitric acid	α -Ketoglutaric acid + CO_2
7. Malic dehydrogenase	Malic acid	Oxaloacetic acid
8. Glutamic dehydrogenase	Glutamic acid	α -Ketoglutaric acid + NH_3
9. Phosphogluconic dehydrogenase	Phosphogluconic acid	Phospho- α -ketohexonic acid
10. Alcohol dehydrogenase	Alcohols	Aldehydes or ketones
11. β -Hydroxybutyric dehydrogenase	β -Hydroxybutyric acid	Acetoacetic acid

CLASSIFICATION OF ENZYMES (Continued)

OXIDIZING ENZYMES (Continued)

Name	Substrate	End Products
C. Dehydrogenases that transfer electrons to cytochrome		
1. Succinic dehydrogenase	Succinic acid	Fumaric acid
2. α -Glycerophosphate dehydrogenase No. 2	α -Glycerophosphate	3-Phosphoglyceric aldehyde
3. Formic dehydrogenase	Formic acid	$\text{CO}_2 + 2\text{H}$
4. Lactic dehydrogenase (from yeast)	Lactic acid	Pyruvic acid
III. Miscellaneous Oxidizing Enzymes		
1. Monamine oxidase, tyramine oxidase	Monamines	Aldehydes + H_2O_2 + NH_3
2. Diamine oxidase (histaminase)	Diamines	Aldehydes + H_2O_2 + NH_3
3. Uricase	Uric acid	Allantoin + CO_2
4. Luciferase	Luciferin	Oxidized luciferin + H_2O_2 + light
5. Dopa oxidase	L-3,4-Dihydroxyphenylalanine	Melanin
6. Glucose oxidase (of fungi)	D-Glucose	Gluconic acid + H_2O_2
7. Lipoxidase	Unsaturated fatty acids	Fatty acid peroxides
8. Fatty acid oxidase	Fatty acids	Acetoacetic acid

DESMOLASES (SPLIT LINKAGES NOT ATTACKED BY WATER)

I. Desmolases requiring cocarboxylase (thiamine pyrophosphate)		
1. Carboxylase	Pyruvic acid	Acetaldehyde + CO_2
2. α -Ketoglutaric carboxylase	α -Ketoglutaric acid	Succinic semialdehyde + CO_2
3. Pyruvic ketolase	Pyruvic acid + acetaldehyde	Acetoin
4. Pyruvic dehydrogenase	Pyruvic acid	Lactic acid + acetic acid + CO_2

11. Isomolases requiring pyridoxal phosphate

A. Amino acid decarboxylases

1. Tyrosine decarboxylase
2. Lysine decarboxylase
3. Arginine decarboxylase
4. Ornithine decarboxylase

B. Transaminases (transfer an amino group from an L-amino acid to a keto acid)

1. Glutamic-aspartic transaminase
2. Glutamic-alanine transaminase
3. Cysteic-aspartic transaminase

III. Other Desmolases

1. Aldolase

2. Oxalacetic decarboxylase
3. Carboligase
4. Phosphorylase

1. Glyoxalase (requires glutathione)

2. Fumarase

3. Aconitase

4. Aldehyde mutase (Cannizzaro reaction)

5. Lactic acid racemase

6. Carbonic anhydrase (contains Zn)

Tyrosine

Lysine

Arginine

Ornithine

from an L-amino acid to a keto acid)

Glutamic acid + oxalacetic acid

Glutamic acid + pyruvic acid

Cysteic acid + oxalacetic acid

Fructose-1,6-diphosphate

Oxaloacetic acid

Pyruvic acid

Glucose-1-phosphate

OTHER ENZYMES

Glyoxals

Fumaric acid

Citric acid

Aldehydes + H₂O

d- or *l*-Lactic acid

H₂CO₃ or carbonates

Tyramine + CO₂

Cadaverine + CO₂

Agmatine + CO₂

Putrescine + CO₂

α -Ketoglutaric acid + aspartic acid

α -Ketoglutaric acid + alanine

Aspartic acid + β -sulfopyruvic acid

Dihydroxy acetone phosphate +
phosphoglyceric aldehyde

Pyruvic acid + CO₂

Diacetyl + H₂O₂

Starch or glycogen + H₃PO₄

Hydroxy acids

l-Malic acid

l-Isocitric acid

Acid + alcohol

dl-Lactic acid

CO₂ + H₂O

liminary work, but the method that Sumner finally evolved for its preparation in crystalline form was very simple. One hundred grams of jack bean meal were stirred with 500 milliliters of 32 per cent acetone and filtered in an ice chest. The urease crystallized from the filtrate after standing overnight in the cold.

Genes and enzymes. Beadle and co-workers have shown that genes are related to enzymes. These workers have modified microorganisms by exposing them to X-rays. After such treatment there are some individuals among the surviving organisms which have lost a chemical mechanism possessed by the parents. The following generations of the modified microorganisms breed true to the characteristics of the mutant strain. In this way new organisms can be developed with biochemical characteristics that differ from the parent strain. From these facts it is postulated that the ability of an organism to synthesize a particular enzyme depends on the presence of a corresponding gene. Thus a descendant would inherit from its parents the ability to synthesize the distinctive collection of hundreds of enzymes which characterize each individual.

FACTORS AFFECTING ENZYME ACTIVITY

The activity of enzymes may be affected by several factors. Such factors include temperature, pH, concentration of substrate, concentration of enzyme, radiant energy, activators, and inhibitors. Although the influence of these factors on enzyme action have been studied almost exclusively *in vitro*, the qualitative, if not the quantitative, effects are probably similar for *in vivo* as well as for *in vitro* reactions.

Temperature. A rise in temperature increases the velocity of reactions catalyzed by enzymes only within definite limits. Since an enzyme is heat sensitive because of its protein nature, two processes are affected by a rise in temperature: (1) an increase in the velocity of the catalyzed reaction due to greater molecular activity, and (2) an increase in the rate of destruction of the enzyme by heat denaturization. Therefore the temperature at which maximum velocity takes place is not necessarily the best temperature for enzyme action since this rapid velocity can be maintained for only a short time.

The optimal temperature for the activity of most enzymes obtained from animal sources is about 37°C , if the time is measured in hours. For the same period the optimum for many plant enzymes lies between 40 and 50°C . If time is measured in days the optimal temperature for both groups of enzymes is much lower and may approximate room temperature. If time is measured in months it is probable that the optimal temperature is between 0 and 10°C , whereas if time is measured in minutes the optimum may be over 60°C .

At 0°C most enzymes are quite inactive. However, fat-splitting enzymes have been found to be active at -15°C . Hence it is possible for fat deterioration to take place in frozen foods unless lipases have been inactivated. Other changes in frozen foods due to enzyme action have been noted when the products have not been treated in such a way as to completely inactivate enzymes.

Prolonged heating at temperatures as low as 40°C will inactivate some enzymes. Most enzymes are destroyed by heating for a few minutes at 80°C , and heating at 100°C inactivates practically all enzymes instantly. This fact is utilized in the preparation of vegetables for canning and freezing. The vegetables are blanched by immersion in hot water or by treatment with steam in order to inactivate enzymes which would otherwise reduce nutritive values or affect colors and flavors.

Hydrogen ion concentration. The activity of an enzyme is greatly affected by the hydrogen ion concentration of its medium.

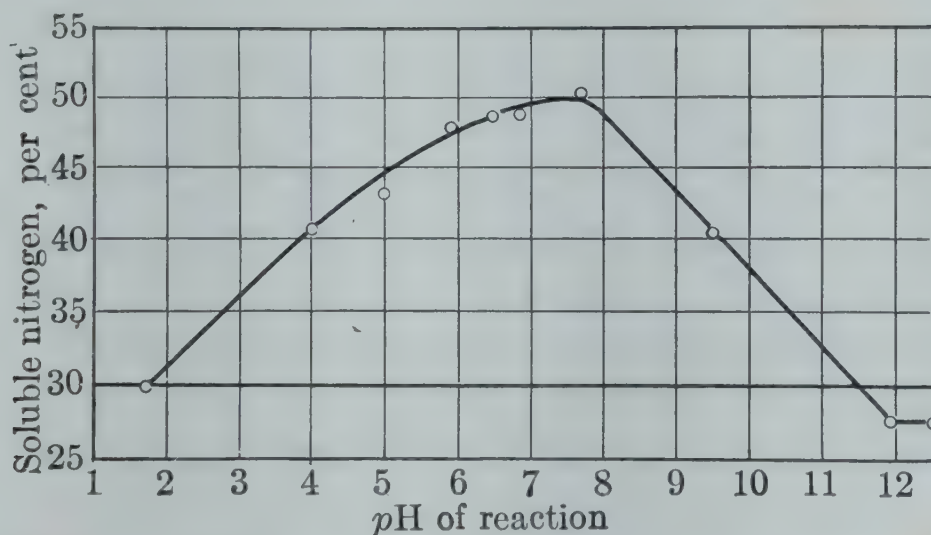


FIG. 6. The effect of $p\text{H}$ on the hydrolysis of soybean protein with papain at 26°C for 1 hour. (From Johnsen and Smith, *Cereal Chem.*, Vol. 25, p. 80, 1948.)

Each enzyme has its optimal pH and pH zone at which it exhibits its greatest activity. This optimum varies widely for different enzymes. Thus pepsin is most active at pH 1.5 to pH 2.0, whereas trypsin shows its greatest activity at about pH 8.0.

The optimal pH is not a fixed value for all conditions but may vary with the source of enzyme, the kind of substrate, the kind of buffer, and the temperature. This is illustrated in the following table:

ENZYME	SOURCE	OPTIMAL pH
Lactase	Almond	4.2
Lactase	Calf Intestine	5.0
Lactase	Yeast	7.0
ENZYME	SUBSTRATE	OPTIMAL pH
Pepsin	Egg albumin	1.5
Pepsin	Casein	1.8
Pepsin	Gelatin	2.0
ENZYME	BUFFER	OPTIMAL pH
Urease	Acetate	6.4
Urease	Citrate	6.5
Urease	Phosphate	6.9
ENZYME	TEMPERATURE	OPTIMAL pH
Malt Diastase	25° C	4.3
Malt Diastase	45° C	5.0
Malt Diastase	60° C	5.7

Concentration of substrate. The initial rate of a reaction catalyzed by an enzyme increases with a rise of substrate concentration up to a certain maximum. This effect is readily explained if we assume the formation of an enzyme-substrate complex. If the substrate concentration is so low that not all the enzyme molecules find substrate molecules with which to combine, the rate of reaction will be increased by the addition of more substrate molecules. When enough substrate is present so that all enzyme molecules are able to react, an increase of substrate concentration will not increase the rate of reaction.

Concentration of enzyme. The velocity of an enzyme-catalyzed reaction is usually directly proportional to the enzyme concentration, when the amount of substrate is not a limiting

factor. This generalization may hold true for only relatively short periods of time, for, unless end products are removed, they will eventually block the reaction.

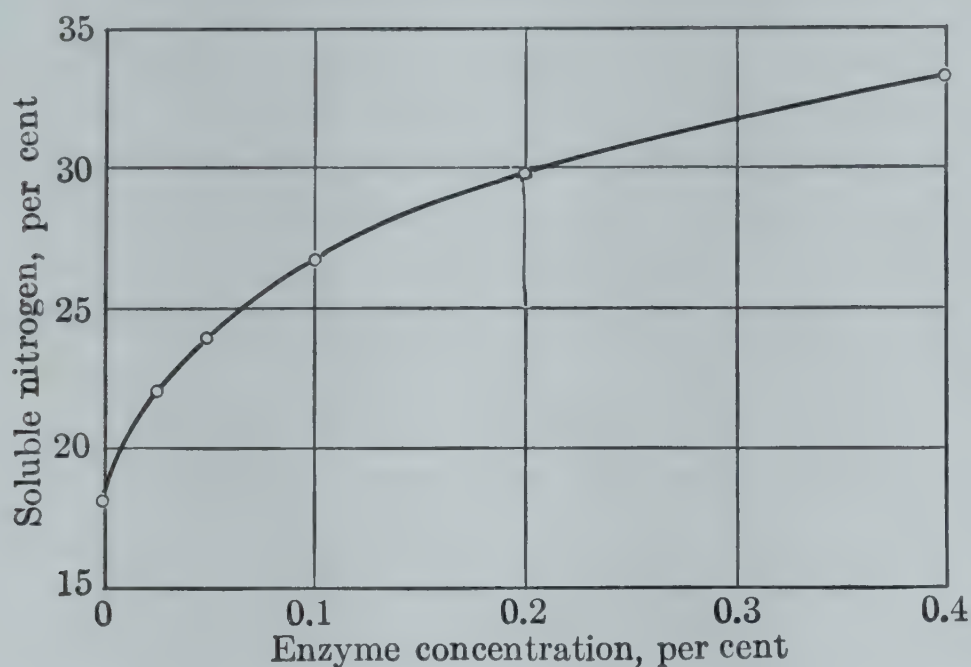


FIG. 7. The relation of concentration of papain to the hydrolysis of soybean protein. Reaction period of 60 minutes, at 26° C, and pH 8.0. (From Johnsen and Smith, *Cereal Chem.*, Vol. 25, p. 81, 1948.)

Radiant energy. Enzymes are usually inactivated by light, the shorter wavelengths having a greater effect than the longer ones. The inactivation of enzymes by ultraviolet rays is to be expected, since proteins are denatured by these rays. The effect of X-rays varies with the type of enzyme; some enzymes are inactivated, whereas others are not affected.

Activators. Enzyme activity is often stimulated by certain chemical substances. Small amounts of such bivalent ions as cobalt, manganese, nickel, and magnesium accelerate the action of many enzymes. The activity of pancreatic lipase is stimulated by bile salts, calcium salts, and albumin. Salivary and pancreatic amylases are activated by chlorides.

Cells sometimes produce enzymes in a completely inactive form. This inactive form is called a *proenzyme* or *zymogen*. When the activator is specific but of unknown organic composition, it is called a *kinase*. An example of such an inactive form is *trypsinogen*, which is produced by the pancreas. When the pancreatic juice containing trypsinogen reaches the small intestine, it meets a substance called *enterokinase* which activates

the zymogen and forms the active enzyme *trypsin*. A kinase differs from a coenzyme since it will not diffuse through a semipermeable membrane and can be inactivated by heating at temperatures under 100°C . Furthermore, once a kinase has activated a zymogen, its work is completed and it is no longer needed for the action of the enzyme.

Inhibitors. Substances that reduce the activity of enzymes are called *inhibitors*. Such substances can be divided into three classes: (1) those that compete with the substrate for an enzyme, (2) those that affect the protein part of an enzyme, and (3) those that affect the non-protein part of an enzyme.

Compounds similar in structure to the natural substrate of an enzyme may become attached to the enzyme and thus prevent its action. Examples of such inhibitors are (1) glucose, which inhibits the action of phosphorylase on glucose-1-phosphate and (2) malonic acid, which inhibits the dehydrogenation of succinic acid to fumaric acid by the action of succinic dehydrogenase.

The second class of inhibitors affect the protein part of an enzyme by reacting with chemical groups such as the sulfhydryl group ($-\text{SH}$), the disulfide group ($-\text{SS}-$), and the amino group ($-\text{NH}_2$) or the phenolic OH group. Inhibitors that attack these groups include ions of heavy metals, and such chemical substances as iodoacetic acid, nitrous acid, formaldehyde, and iodine.

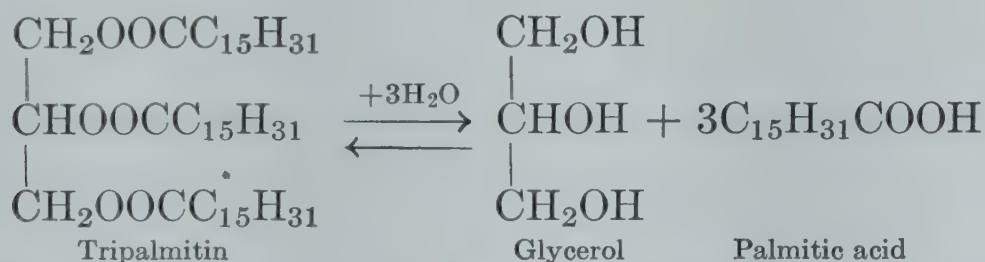
The third group of inhibitors, affecting the non-protein part of enzymes, are substances such as (1) CO , H_2S , HCN , and NaN_3 , which inhibit the iron-porphrin enzymes, (2) H_2S , HCN , and diethyldithiocarbamate, which inactivate enzymes containing copper, and (3) fluorides and oxalates, which inhibit enzymes requiring calcium.

Living organisms apparently produce substances that inhibit enzyme action. Such substances are called *antienzymes*. For example, certain antienzymes protect the stomach and intestine from the action of proteolytic enzymes which are active in these organs. Antienzymes are also believed to be present in parasites that live in the intestine and are able to withstand the action of digestive enzymes. Antipepsin, antitrypsin, antirenin, and anticatalase are some of these mysterious agents.

ESTERASES

Esterases are hydrolytic enzymes which split esters into their respective acids and alcohols. Most esterases will catalyze the synthesis as well as the hydrolysis of their substrates. Lipases, phosphatases, polynucleotidases, and other esterases occur in plants and animals. Pectase, tannase, and chlorophyllase occur only in plants, and choline esterase and cholesterase occur only in animals.

Lipases. Lipases catalyze the hydrolysis and synthesis of fats and oils.



Plant lipases are found in greatest amounts in oil-bearing seeds such as castor bean, soybean, flax, corn, cottonseed, coconut, rape, hemp, mustard, and poppy. The action of seed lipases is not limited to the oils of plants but also takes place with animal fats as substrates.

Pancreatic lipase, the best-known animal lipase, is present in both the pancreas and pancreatic juice. Liver and muscle tissues also contain esterases which hydrolyze fats. These enzymes show greater activity with simpler compounds such as the ethyl esters of fatty acids than they do with fats or oils as substrates.

Most of the studies concerning the characteristics of plant lipases have been made with castor bean lipase (ricinus lipase), since the most active preparation can be obtained from these seeds. The enzyme as obtained from the fat-free bean is inactive and must be treated with acid to activate it. Pancreatic lipase (steapsin) is activated by the addition of calcium salts. Bile salts also accelerate the action of pancreatic lipase by emulsifying the substrates, thereby furnishing greater opportunity for contact between enzyme and substrate. The optimal hydrogen ion concentration for the action of ricinus lipase is about $p\text{H}$ 5.0, whereas the optimum for pancreatic lipase lies between $p\text{H}$ 7.0 and $p\text{H}$ 9.3.

CARBOHYDRASES

Carbohydrases are found in most forms of plant and animal life. The reversibility of the action of these enzymes is difficult to prove, and at present we do not know with certainty which carbohydrases can catalyze the synthesis as well as the hydrolysis of their substrates. It is probable that neither starch nor glycogen can be formed by the action of amylases, whereas the hydrolysis of these compounds proceeds rapidly.

Amylases. Amylases are enzymes that hydrolyze starch, glycogen, and dextrins. Granular starch is hydrolyzed very slowly by amylases in vitro. Starch, ground in a pebble mill, is hydrolyzed more rapidly than granular starch, and boiled starch paste is digested very quickly. Wheat amylase, described by Kirchoff in 1811, was probably the first enzyme to be discovered. Many other sources of these carbohydrases have been found since the discovery of amylase in wheat. They are among the most widely distributed of all enzymes, being found in most higher plants and animals and in many microorganisms. Large amounts occur in the pancreas of animals, in germinating seeds, in several bacteria, and in many fungi. The commercial preparation, Taka-diastase, which is used in medicine and in analytical chemistry, is obtained from the fungus *Aspergillus oryzae*. Commercial preparations and enzyme mixtures called *diastases* contain α -amylase, β -amylase, phosphatase, maltase, and small amounts of other carbohydrases.

α -Amylase. α -Amylase hydrolyzes starch and glycogen to dextrins. Since by this action starch gels are liquefied, this enzyme is called the *liquefying* or *dextrinogenic* amylase. α -Amylase occurs in animal tissues, fungi, and germinated grains. It is not present in an active form in ungerminated seeds. Pancreatic and salivary amylases contain α -amylase. Its separation from β -amylase has been accomplished by heating a crude mixture of the two at 70° C for 15 minutes. The β -amylase is completely inactivated while the more heat-stable α -amylase retains much of its activity.

The principal products of the action of α -amylase on starch are low molecular weight dextrins composed of from four to

eight glucose units. Such dextrans have reducing powers but give no color when treated with iodine. These low molecular weight dextrans contain 1,6- as well as 1,4-glucosidic linkages. Maltose is produced very slowly from starches and dextrans by α -amylase. From these facts the action of α -amylase has been postulated as taking place on *amylopectin*, the branched-chain component of starch. Only α -1,4-glucosidic linkages are split as shown in Fig. 8.

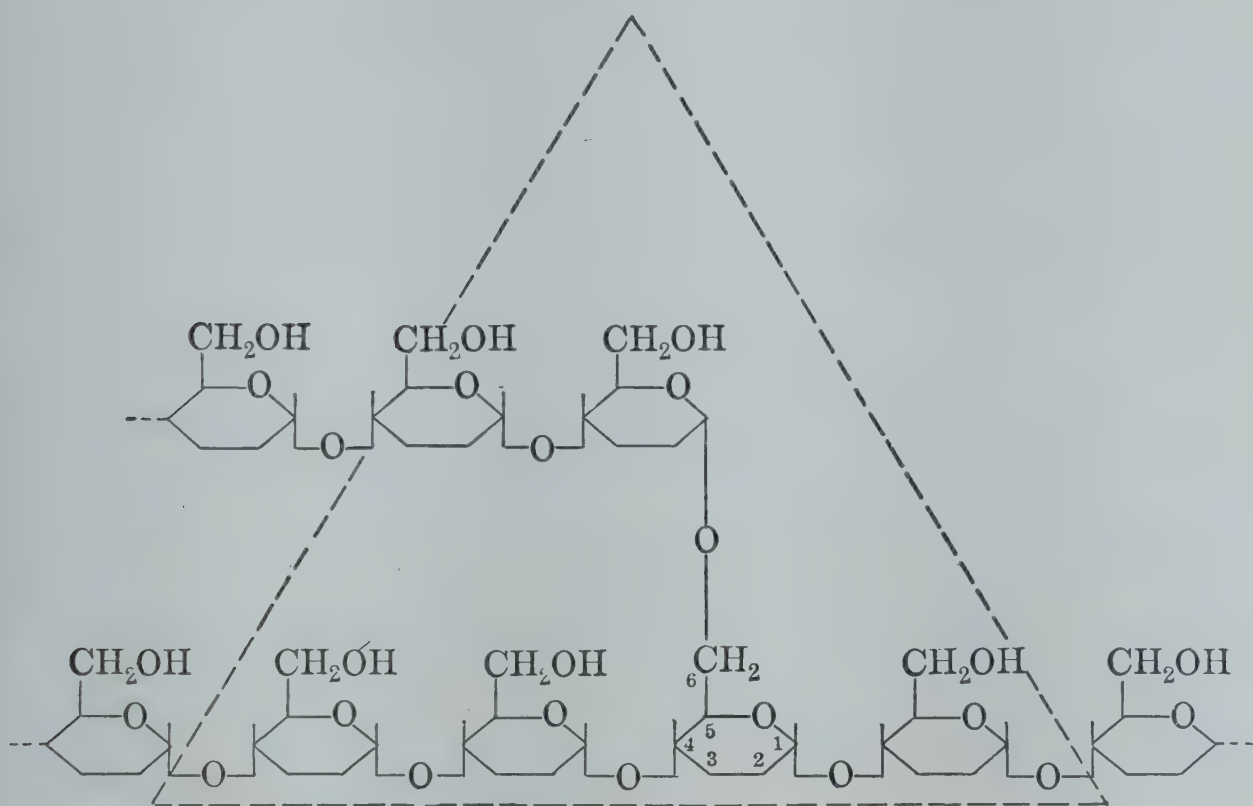


FIG. 8. Section of an amylopectin molecule. α -Amylase splits 1,4 linkages, forming a small dextrin such as the one shown within the triangle.

β -Amylase. This amylase hydrolyzes starch, glycogen, and certain dextrans to maltose. Since the characteristic end product of the reaction is a sugar, the enzyme is described as the *saccharogenic* amylase.

β -Amylase is found in relatively high concentrations in ungerminated barley, wheat, and rye. It is present in the germinating seeds of these cereals in even larger amounts. This amylase is also present in soybeans, in sweet and white potatoes, and probably in many other plants. A large number of plant sources of amylases have not been investigated in such a way as to differentiate between the presence of α - and β -amylases.

The amylose fraction of starch is completely hydrolyzed by

β -amylase to maltose. Amylopectin is hydrolyzed to maltose and to high molecular weight dextrans which retain many of the properties of starch. This mixture of starchlike dextrans is called *residual dextrin*. These starchlike dextrans are non-reducing and give a blue-violet color with iodine. The failure of β -amylase to completely hydrolyze amylopectin as it does amylose is explained by the fact that this enzyme cannot hydrolyze the 1,6-glucosidic linkages. Maltose is split from the ends of amylopectin branches, by cleavage of 1,4-glucosidic linkages, until a 1,6-linkage occurs. At this point its action ceases. If α -amylase is present to remove these linkages, the β -amylase will continue its action on the resulting end groups.

Sucrase. Sucrase is the enzyme which hydrolyzes sucrose to glucose and fructose. This enzyme is also called *invertase* or *saccharase*.

There are two enzymes that can hydrolyze sucrose. The first, *glucosucrase*, hydrolyzes sucrose by attacking the glucose part of the molecule. The second, *fructosucrase*, hydrolyzes sucrose by attacking the fructose part of the molecule. The difference between the two sucrase enzymes is shown by their action on raffinose. Raffinose is composed of three hexose units linked in the order galactose-glucose-fructose. Glucosucrase does not hydrolyze raffinose since no glucose end group is present for its action. However, fructosucrase attacks raffinose with the formation of fructose and melibiose.

Fructosucrase, also known as β -*fructofuranosidase*, is found in plants, microorganisms, and some invertebrates. Yeast is particularly rich in this enzyme. Several fungi, many bacteria, and blossoms, leaves, and fruits of most plants contain fructosucrase. It occurs in the stems and leaves but not in the roots of sugar beets which contain large amounts of sucrose. Fructosucrase is not present in higher animals. The invertase in animal tissues seems to be glucosucrase.

PROTEINASES

Enzymes that catalyze the hydrolysis of proteins are called proteinases. The products of hydrolysis are proteoses, peptones, polypeptides, and small amounts of amino acids. Most pro-

teinases have the property of hydrolyzing a number of closely related compounds which include native proteins, denatured proteins, proteoses, and peptones. These enzymes seem to be present in all plant and animal tissues that metabolize proteins.

The exact types of linkages hydrolyzed by each of the proteinases is still unknown. It is known that such proteolytic enzymes as pepsin and trypsin act differently on the same protein. Since only small amounts of free amino acids are liberated, it is believed that the proteinases do not hydrolyze terminal peptide linkages to any great extent but attack those that are more centrally located.

Most of the proteinases, with the exception of cathepsins, are exoenzymes. Thus, enzyme preparations with proteolytic action can be made from the bran on which certain molds have been grown.

The proteinases can be divided into four classes:

1. *Proteinases that are most active in acid media.* The common example of this type is pepsin, which is found in the gastric juice of mammals, birds, reptiles, and fish. It is secreted by the gastric mucosa as the inactive proenzyme *pepsinogen* which is converted to the active pepsin by contact with acid. A second enzyme active in acid media is *rennin*. This is found in the stomach of young animals such as the calf and the lamb where its function is to clot milk. As the animal grows older, rennin gradually disappears and its function is taken over by pepsin. Rennin, like pepsin, is secreted as an inactive zymogen or proenzyme and is activated by the action of acid.

2. *Proteinases that are most active in alkaline or neutral media.* An example of this group is *trypsin*. The zymogen *trypsinogen*, found in the pancreatic juice, is activated by *enterokinase* in the small intestine to form trypsin. Probably all animals have a proteolytic enzyme of this type. The insect-digesting enzyme of the "pitcher" plant belongs to the group.

3. *Proteinases that are activated by reducing agents such as cysteine and glutathione.* These enzymes are called *papainases* and are found only in plants and microorganisms. The green papaya fruit contains a large amount of the enzyme *papain*. This has been the cheapest commercial proteolytic enzyme. The sap of the fig tree contains *ficin* which has been used for many years

to kill and digest certain intestinal worms. *Bromelin* is found in pineapples. Its presence in fresh pineapple prevents the setting of gelatin desserts made with this fruit. Papainases are also present in wheat and in seeds of other grains.

4. *Proteinases that are not secreted by cells and are not activated by reducing agents.* These endoenzymes are called *cathepsins*. They are probably present in the cells of all animal tissues. During life, cathepsins are inactive due to an unfavorable hydrogen ion concentration. Tissues become more acid after death and cathepsins become active, resulting in the hydrolysis of proteins of the cells. One commercial meat tenderization process is accomplished by the action of these enzymes. If ultraviolet radiation is used to prevent the surface growth of bacteria and molds, meat may be kept in an atmosphere of high humidity at room temperature without rapid spoilage. Three days' storage at 15° C is then sufficient to tenderize meat adequately. At temperatures of 3° C meat must be aged for several weeks to achieve a similar result.

PEPTIDASES

Peptidases hydrolyze peptides of various sizes from dipeptides to the complex polypeptides of partially digested proteins.

Dipeptidase which splits only dipeptides is found in animals, plants, and microorganisms. It occurs in such diversified materials as kidney, leucocytes, milk, malt, and yeast. It is one of a mixture of enzymes which was formerly thought to be a single peptidase called *erepsin*.

Carboxypolypeptidase (sometimes called carboxypeptidase) acts on peptides, splitting off the amino acid having the free carboxyl group. Carboxypeptidase occurs in the pancreas of higher animals as the inactive zymogen, and apparently it is activated by trypsin. This enzyme is a constituent of the erepsin complex.

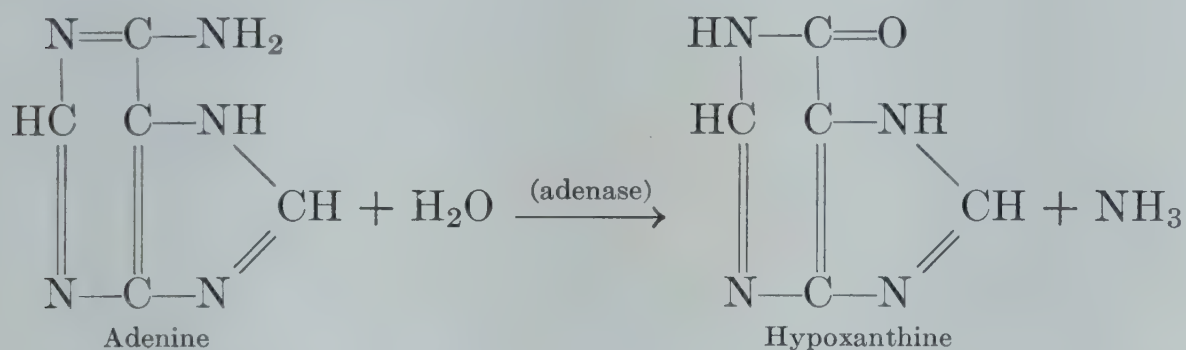
Aminopolypeptidase readily hydrolyzes tri- and tetrapeptides of naturally occurring amino acids but also attacks longer peptides. When these substrates contain a free amino group, the amino acid bearing this group is split from the peptide. Amino-

polypeptidase occurs in yeast, intestines, kidneys, spleen, liver, and other tissues.

Other peptidases found in nature prefer or require substrates whose peptide chains start with a particular amino acid such as proline or leucine.

AMINASES

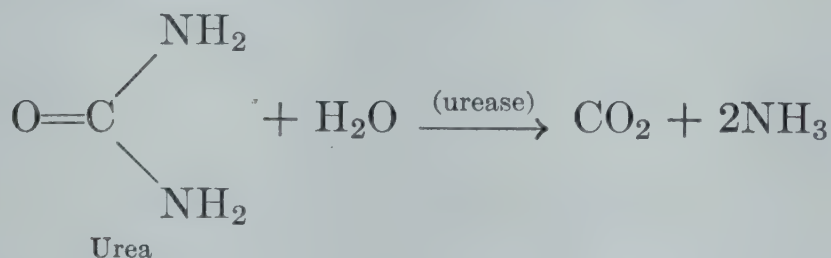
Enzymes that hydrolyze amines are called *aminases* or *deaminases*. An example is *adenase* which hydrolyzes adenine to hypoxanthine and ammonia.



Adenase is found in cow's muscle and cow's milk but has not been found in human tissues. Certain aminases break down such substrates as adenosine, adenylic acid, cytidine, guanine, arginine, aspartic acid, and other amines.

AMIDASES

The amidases split amides. *Urease* is an example of this group of enzymes. Urease occurs in many species of bacteria,



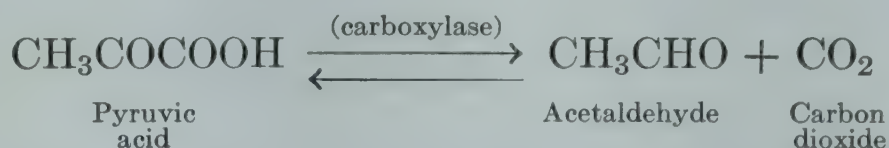
yeast, and fungi, in the gastric mucosa and kidneys of animals, and in higher plants. Beans are a particularly rich source of this enzyme. Urease is specific for urea and does not have

the property of hydrolyzing other substrates. Other amidases split such compounds as allantoin, asparagine, glutamine, and histidine.

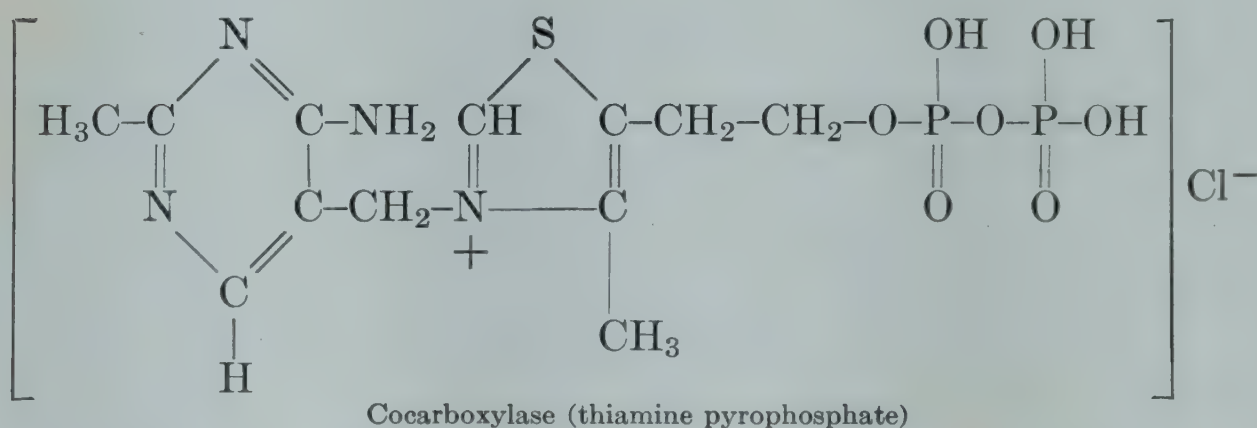
DESMOLASES

Enzymes that break linkages that are not attacked by water are called desmolases. These enzymes split C—C and C—N and other bonds.

Carboxylase. In 1910, Neubauer suggested that ethyl alcohol resulting from yeast fermentations was formed from pyruvic acid. He postulated that pyruvic acid was decarboxylated to acetaldehyde, which was then reduced to ethyl alcohol. This mechanism was later proved to be correct. The first step in this proof was the discovery of an enzyme in yeast which could decarboxylate pyruvic acid. It has been found in bacteria, fungi, higher plants, and animals, as well as in yeast.

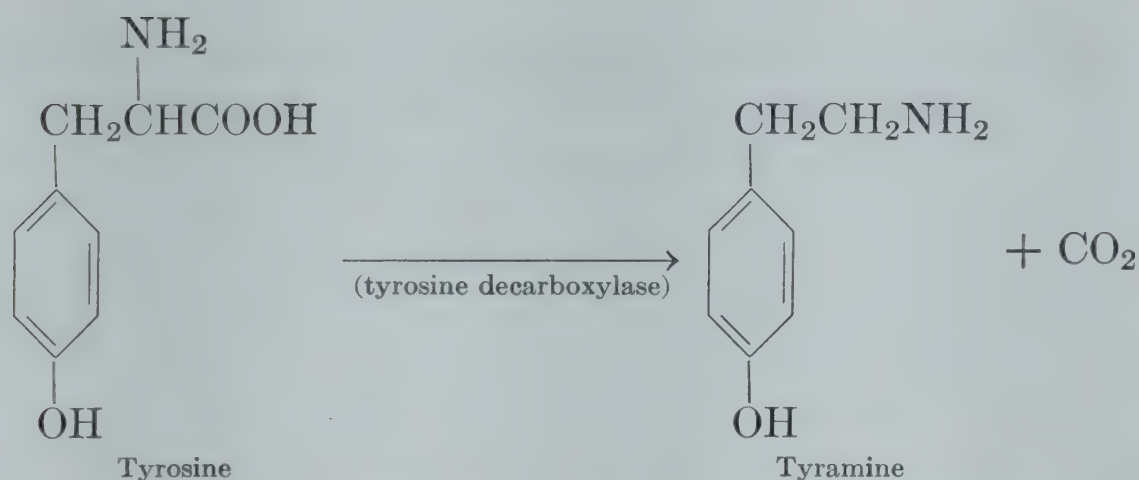


Carboxylase requires a divalent ion, such as magnesium or manganese, and a coenzyme for its action. This coenzyme (cocarboxylase) is thiamine pyrophosphate, a compound of vitamin B₁.

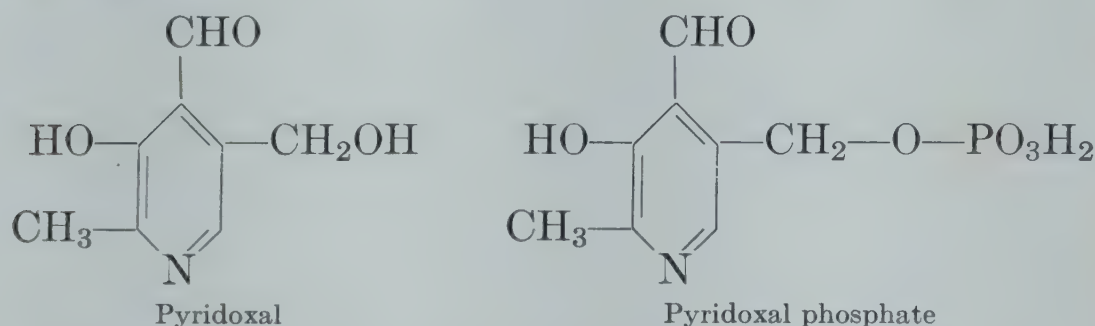


Cocarboxylase (thiamine pyrophosphate)

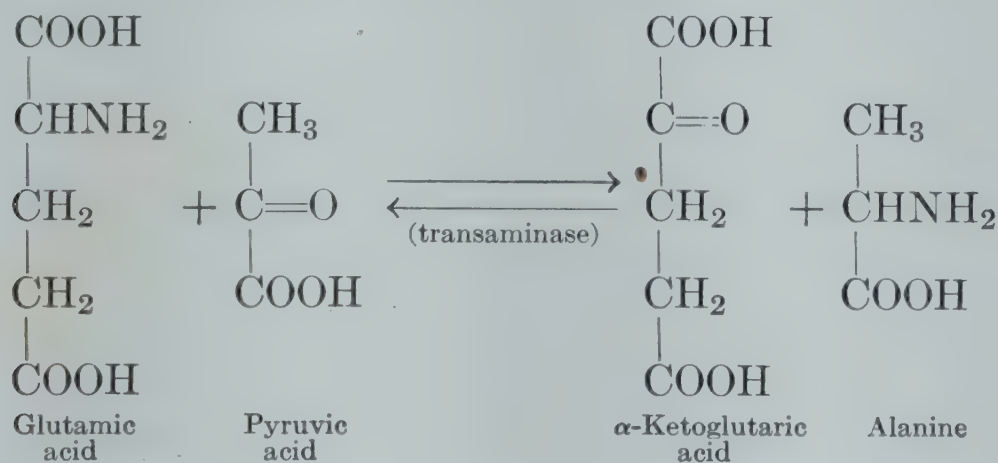
Amino acid decarboxylases. Many bacteria can decarboxylate amino acids to amines. These bacteria contain *amino acid decarboxylases*, seven of which have so far been identified. An example of their action is the formation of tyramine from tyrosine.



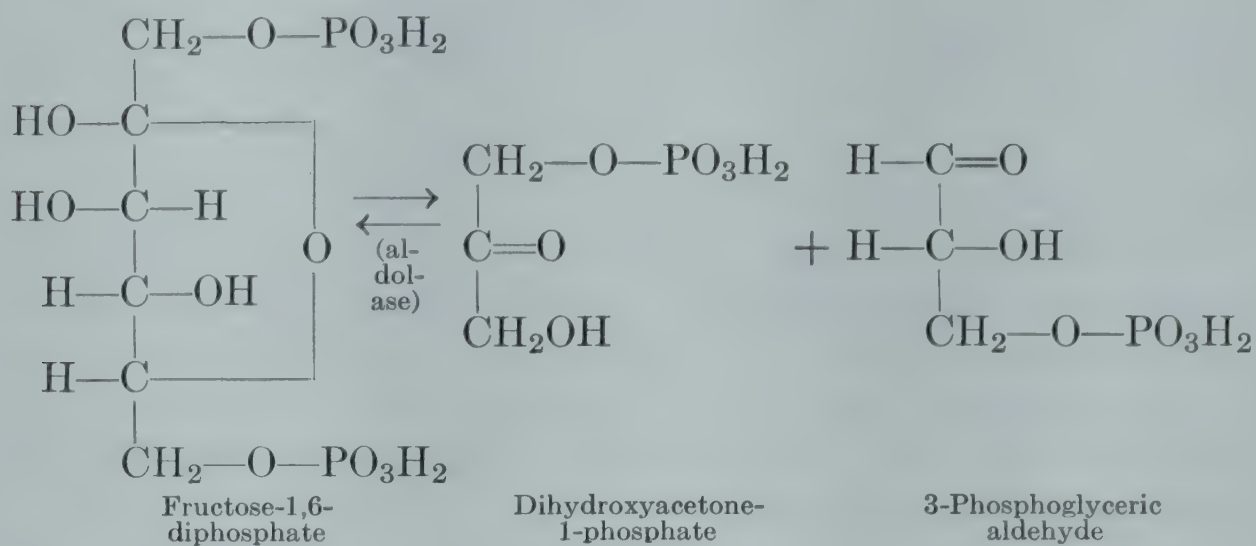
In addition to tyrosine decarboxylase, lysine, glutamic acid, arginine, ornithine, dihydroxyphenylalanine, and histidine decarboxylases are known. All these enzymes except the one acting on histidine require pyridoxal phosphate as a coenzyme. Pyridoxal phosphate is a derivative of vitamin B₆, pyridoxine, and is called *codecarboxylase*.



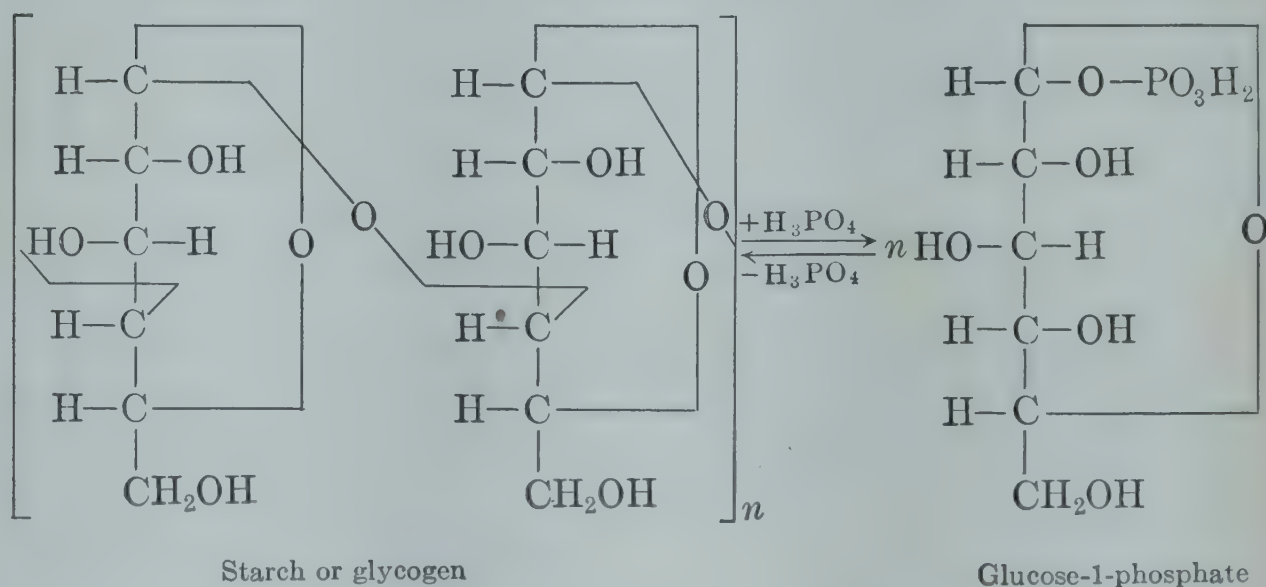
Transaminases. One of the interesting reactions of amino acids in living matter is the transfer of amino groups from one compound to another. Enzymes called *transaminases* catalyze the transfer of amino groups from amino acids to keto acids. These catalysts are found in practically all animal tissues, in bacteria, and in higher plants. Transaminases require pyridoxal phosphate as a coenzyme. An example of transamination is the reaction between glutamic acid and pyruvic acid:



Aldolase. A desmolase that probably occurs in all cells is the enzyme that splits fructose-1,6-diphosphate into two triose phosphates. This enzyme is *aldolase*, and it catalyzes the following reaction:



Phosphorylase. In 1947 Carl Cori and Gerty Cori won a Nobel prize for their isolation and study of phosphorylase. Subsequent work has shown that more than one phosphorylase exists; therefore the enzyme studied by the Coris is sometimes called *glucosanphosphorylase*. The reaction catalyzed is the first step in the transformation of starch or glycogen to compounds that are oxidized to carbon dioxide and water by living cells. In the presence of phosphate, phosphorylase transforms starch or glycogen to glucose-1-phosphate. The reaction is reversible if traces of dextrans or glycogen are present.



Phosphorylase is found in animals, plants, and microorganisms. It occurs in muscle, heart, liver, and brain; peas, beans, and potatoes are good sources of phosphorylase in higher plants.

We have discussed only a few of the many hydrolytic and desmolytic enzymes. The reader is referred to the list of reference books at the end of this chapter for more detailed discussions of these and other enzymes. Oxidizing enzymes will be discussed in the chapter on biological oxidation.

PRACTICAL APPLICATIONS OF ENZYMES AS CATALYSTS

Enzymes were used as catalysts for various reactions for hundreds of years before they were recognized as definite chemical substances. The description of the agents (*ferments*) which produced fermentations precedes our knowledge of the existence of enzymes in living cells. Processes such as bread-making, brewing, alcohol manufacture, and wine-making have been known from antiquity.

Industrial applications. One of the older applications of enzymes which is still employed is the use of rennin or rennet to make cheese. The enzyme coagulates the casein of milk, forming insoluble calcium paracaseinate. Commercial rennet is prepared from the fourth or true stomach of the calf. Preservatives such as boric acid, benzoic acid, and large amounts of sodium chloride are sometimes added to prevent decomposition of the enzyme preparations by bacteria. Most of the millions of pounds of cheese produced annually in the United States is made with the aid of rennet.

The manufacture of fruit juices has grown from a very small industry twenty years ago to a relatively large industry. This growth has been due in part to the ability of the producers to clarify juices with the aid of enzymes. A mixture of pectic enzymes is added to the juice to hydrolyze pectic substances which cause turbidity.

All woven fabrics contain starch or other sizing applied to the warp threads to strengthen the yarn before weaving. If the woven fabrics are to be bleached, printed, or dyed, this sizing must be removed. The starch can be removed by hydrolysis with

amylases. An enzyme method of desizing is more satisfactory than a method employing acid or alkali because these latter agents attack cellulose and weaken the fabric. Several enzyme products for desizing cloth are manufactured in the United States. These preparations are made from bacteria, fungi, malt, or other biological source materials.

In the manufacture of leather, two steps in the process require the action of enzymes. Proteolytic enzymes prepared from such materials as the pancreas or *Bacillus mesentericus* are used to hydrolyze the proteins of the hair follicles, thus freeing the hair so that it may be readily scraped from the hides. Similar proteolytic enzyme products are employed in a second stage, called bating, as a preparation for tanning.

Enzymes are used in dry cleaning for the removal of stains caused by glue, gelatin, or starch. Amylases are employed to prepare a partially hydrolyzed starch as surface coating for paper. Pepsin is used to digest gelatin in the process of recovering silver from photographic film.

Processes that employ microorganisms for the production of specific chemical substances are, in effect, an application of enzymology. Such processes produce lactic acid, acetic acid, citric acid, gluconic acid, gallic acid, L-sorbose, acetone, butanol, ethyl alcohol, and many other products.

Medical applications. Digestive disturbances due to an insufficiency of enzymes have been treated for many years by supplying the lacking enzymes. Pepsin, papain, bromelin, and amylases aid digestion in the mouth and stomach. Pancreatic enzymes can be supplied by encapsulating them in material that is not dissolved in the stomach but is soluble in the juices of the duodenum. The enzymes are not released, therefore, until they arrive at the site of action.

Sloughing wounds and abnormal conditions such as bed sores, furuncles, ringworm, and other suppurative skin diseases have been alleviated by means of proteolytic enzymes from the pig pancreas. The enzymes apparently digest proteolytic matter which prevents the healing of such wounds.

A sterile papain hydrolyzate of meat placed in a solution of salt, glucose, and vitamins has been used in the treatment of starvation in India. Similar preparations of protein hydrolyzates

are now becoming increasingly important in feeding malnourished cases, in certain types of postoperative cases, and in diseases of the digestive tract.

Analytical applications. Mixtures of amylases and maltase, called *diastases*, are used in analytical chemistry for starch determinations. The method employed measures the increase in reducing sugars resulting from the enzymic hydrolysis of starch. This method is more specific than one that measures the increase of reducing sugars after hydrolysis by acid.

Uricase, the enzyme that converts uric acid to allantoin, is used in a method for the determination of uric acid in blood. Urease is employed for the determination of urea in blood and urine. The ammonia formed is determined either by titration or by the color formed with Nessler's solution.

Sucrose and raffinose are determined in mixtures of sugars and in sugar products by polarization before and after treating solutions with sucrase and melibiase. The activity of phosphatase present in milk is used as a measure for the efficiency of pasteurization. The inactivation temperature and time needed to destroy phosphatase activity is such that the most resistant pathogenic bacteria in milk will be killed before the enzyme is completely inactivated.

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- SUMNER, J. B., and G. F. SOMERS. *Chemistry and Methods of Enzymes*. Academic Press, New York, 1947 (2nd edition).
- TAUBER, H. *Chemistry and Technology of Enzymes*. John Wiley and Sons, New York, 1949.

8 • Biological Oxidations

The most fundamental reactions of life are the production of energy-rich foods from carbon dioxide and water and the controlled liberation of this energy for the use of living cells. The first of these processes is accomplished by green plants through a chemical reduction of carbon dioxide. The second process, the liberation of stored energy, is a result of the biological oxidation of foods or of compounds derived from foods.

The principal sources of energy for both plants and animals are carbohydrates, fats, and proteins. These compounds are relatively stable and, with the exception of fatty compounds, do not oxidize spontaneously outside living cells. But living cells readily oxidize these compounds with the aid of enzymes, thus liberating heat and other forms of energy. The energy that is not liberated as heat is the useful source of energy for the cell.

There are probably several systems by which biological oxidations take place in cells, but only one of these systems has been studied in detail. In this one known system biological oxidation has been shown to be a complicated process catalyzed by several enzymes in a connected series of reactions. The substrates for this known system are those compounds which arise from carbohydrate metabolism. Several of the products formed during carbohydrate metabolism are also produced by the breakdown of fats and proteins. Thus a common series of compounds has been found which can arise from any of the major components of foods and which act as substrates in biological oxidation. These compounds, known as components of the *Krebs citric acid cycle*, will be discussed later in this chapter.

Before we examine the reactions and enzymes of biological oxidations, let us review the fundamentals of oxidation and reduction. A principle that should be remembered is that when-

ever one substance is oxidized another is reduced. Oxidations involve one or more of the following changes:

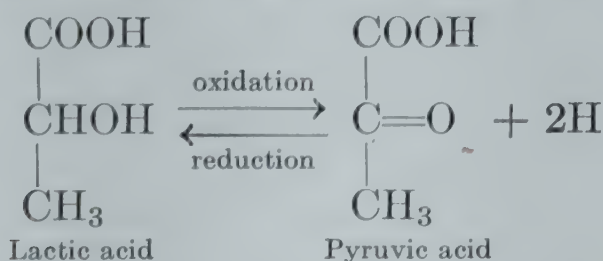
1. *Gain of oxygen.*

Example: Carbon is oxidized to carbon dioxide.



2. *Loss of hydrogen.*

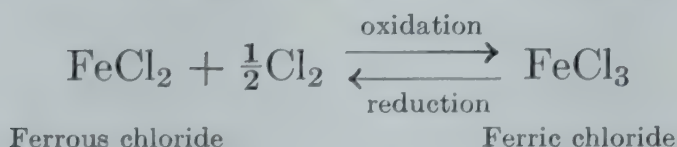
Example: Lactic acid is oxidized to pyruvic acid.



This typical biological oxidation will take place only with the aid of lactic acid dehydrogenase.

3. *Gain of positive valency.*

Example: Ferrous iron is oxidized to ferric iron.



4. *Loss of electrons.* The modern theory explains any oxidation as a reaction in which there is a loss of electrons by the substance oxidized. Thus, in example 1, carbon loses four electrons to oxygen to become C^{++++} . The electrons are not removed any great distance from their original site on the carbon atom but they have moved away. Whether the distance of the electrons from their original orbits on the carbon atom is increased 10 angstrom units (1×10^{-7} centimeter) or 10 feet, the resulting loss of electrons is an oxidation. The second example is readily understood. Two hydrogens, each with an electron, are removed from lactic acid, resulting in a loss of two electrons. In the third example ferrous iron, Fe^{++} , loses an electron to chlorine and becomes Fe^{+++} . These three examples illustrate the fact that all oxidations have one common feature, i.e., a loss of electrons by the substance oxidized.

Biological oxidation. The general equation for the oxidation of food components in biological systems can be written as follows:



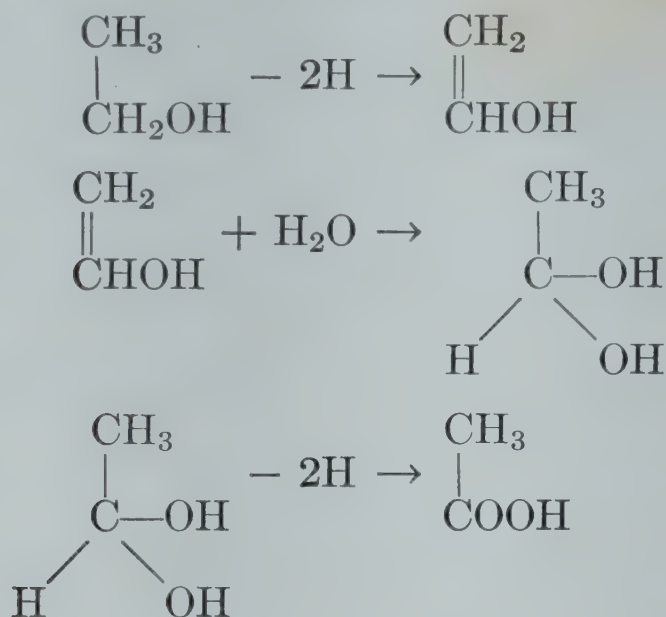
In attempts to explain the mechanism of this reaction two theories have been advanced. These are called the Warburg theory and the Wieland theory. However, the theories of Warburg and Wieland fail to explain the production of carbon dioxide. A third theory, explaining the formation of carbon dioxide, supplements the previously mentioned theories.

The Warburg theory. Since food components, such as glucose, are quite stable to oxidation outside of cells but are rapidly oxidized within living cells, it seemed probable to pioneer workers that the cell must be able to activate either the food material or the oxygen. However, no active foodstuff had been discovered, whereas active oxygen such as ozone and atomic oxygen was well known. Thus it seemed logical that biological oxidations proceeded by an activation of oxygen.

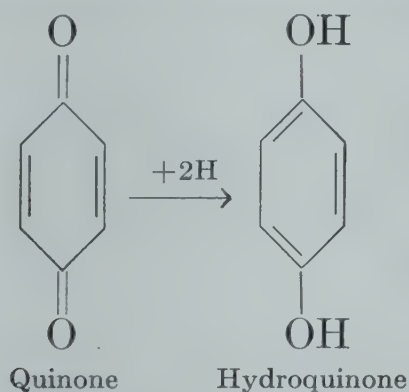
Warburg found that charcoal prepared from hemin, an iron compound, would catalyze the reaction between atmospheric oxygen and certain metabolites. Ordinary charcoal prepared from sucrose possessed no such catalytic effect unless iron was added. Warburg and his colleagues also found that the respiration enzyme of yeast was similar to hemoglobin, which also contains iron, particularly with respect to its reactions with carbon monoxide. He concluded that (1) the respiratory enzyme was an iron compound related to hemoglobin, (2) the primary reaction of respiration was the reaction between molecular oxygen and the iron part of the enzyme, (3) the enzyme produced active oxygen, and (4) active oxygen, not molecular oxygen, reacted with organic metabolites. Later, Keilin and co-workers showed that Warburg's respiration enzyme was similar to, if not identical with, cytochrome oxidase, a catalyst found in most living cells.

Wieland theory. Wieland performed some interesting experiments in which he showed that biological oxidations could be *dehydrogenations*. He studied the oxidation of ethyl alcohol to acetic acid in wine. This reaction takes place readily if the wine is exposed to air. But Wieland found that this oxidation would also take place in the absence of air if quinone was added. He interpreted this as showing that the quinone acted as a hydro-

gen acceptor and that the oxidation was actually a dehydrogenation which took place as follows:



The source of the additional oxygen atom in acetic acid is a molecule of water. The hydrogen given off in the above reaction is accepted by quinone which is reduced to hydroquinone.



Wieland studied several biological oxidations and concluded that all of them were essentially dehydrogenations.

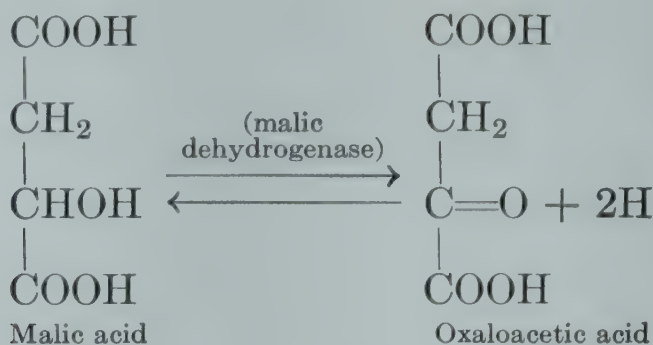
Production of CO_2 . One of the characteristics of aerobic bio-oxidation (*respiration*) is that carbon dioxide is produced. The principal known mechanism by which carbon dioxide is produced by respiring cells is through the decarboxylation of organic acids. One example of an enzyme which catalyzes such a reaction, carboxylase, was discussed in the previous chapter. Several other enzymes capable of catalyzing such decarboxylations are known. The substrates are either keto acids or amino acids.

General mechanism of biological oxidation. The following concept, including the three ideas just discussed, has now been evolved:

1. Food material is changed, with the aid of a series of hydrolytic and other enzymes, from large molecules to compounds with fewer atoms, the actual metabolites which are oxidized.

2. The oxidizable compounds are principally salts of organic acids such as succinic, malic, and isocitric acids.

3. The oxidation of the organic acid metabolites takes place by dehydrogenation. Succinic acid becomes fumaric, malic acid is changed to oxaloacetic, and isocitric acid is oxidized to oxalosuccinic acid.



4. Oxidation takes place by the removal of 2H from each substrate, i.e., two protons (2H^+) and two electrons ($2e$) are removed.

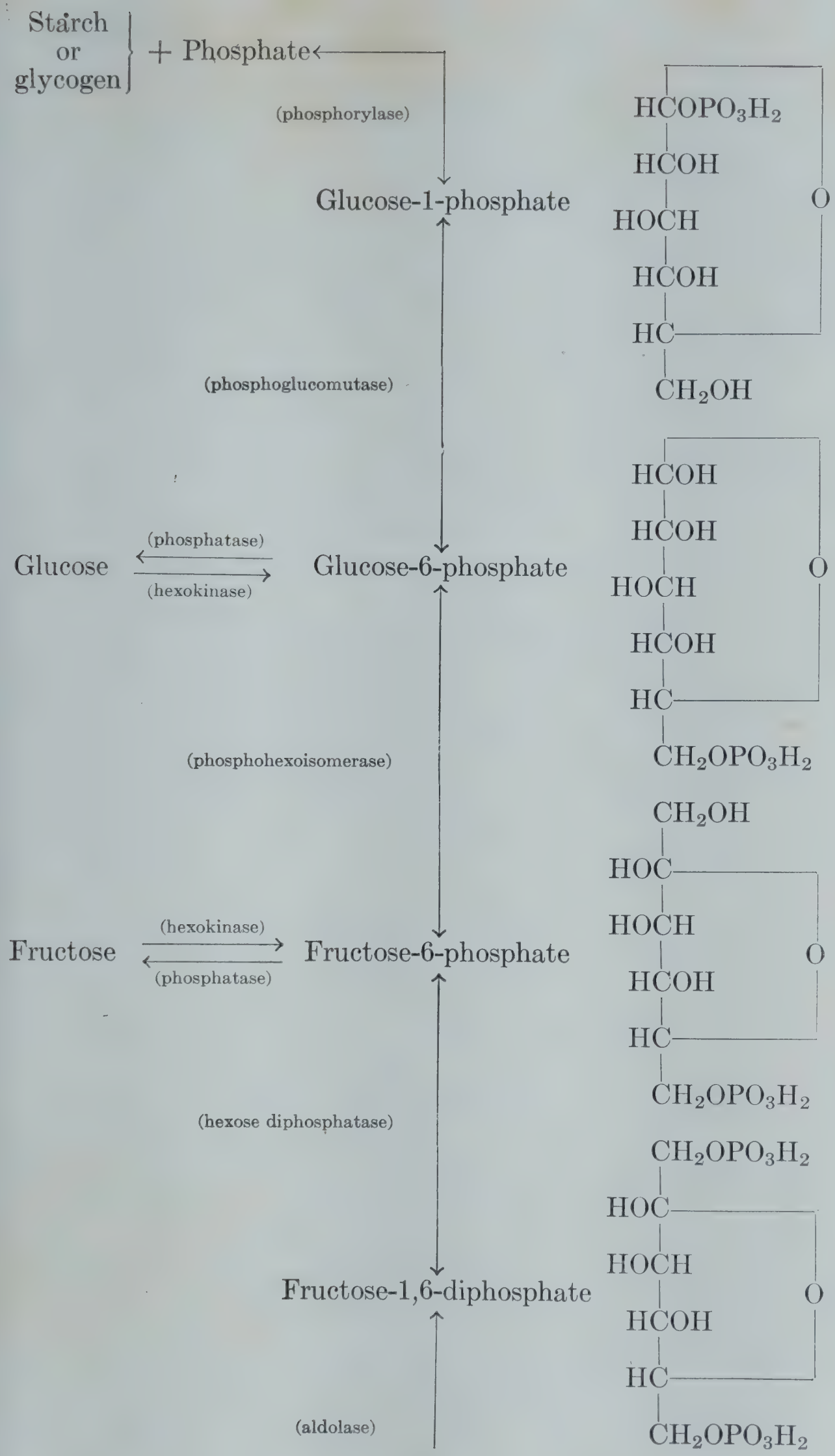
5. The protons and electrons that are removed from the metabolites are transferred through a series of carriers. At each transfer of electrons a small amount of energy is liberated.

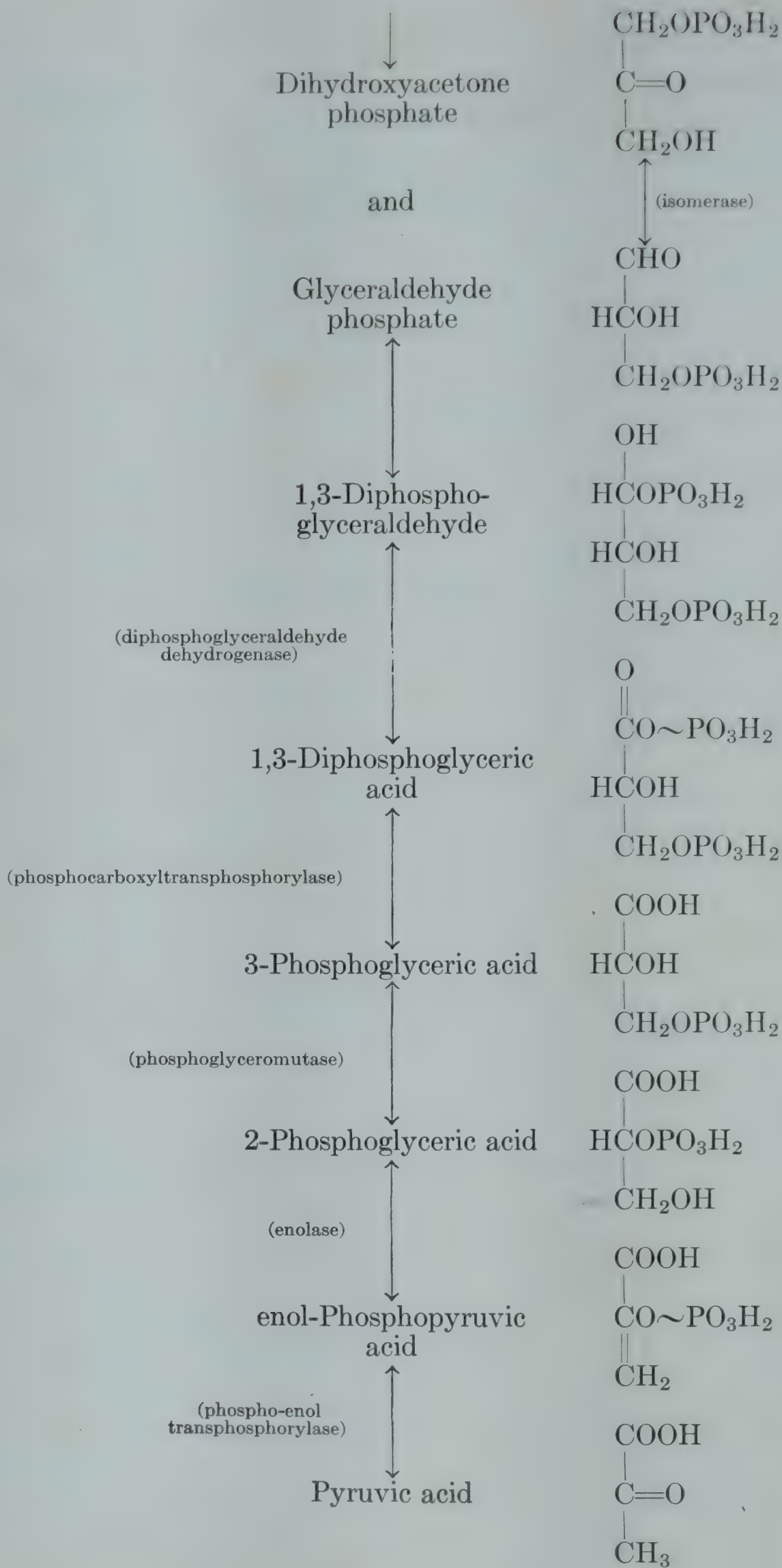
6. The final transfer of electrons and protons is made to molecular oxygen with the aid of the oxidizing enzyme called cytochrome oxidase. Water is formed as the final product.

7. Carbon dioxide is produced in the process by the decarboxylation of such acids as oxalosuccinic, α -ketoglutaric, and oxaloacetic.

INTERMEDIARY STEPS IN CARBOHYDRATE METABOLISM

The metabolism of carbohydrates in living organisms has been studied intensively and is much better understood than is the metabolism of lipids and proteins. Starch, glycogen, glucose, or fructose are changed through a series of phosphorylated intermediates to pyruvic acid. These changes are catalyzed by enzymes. The following chart shows the changes that take place.

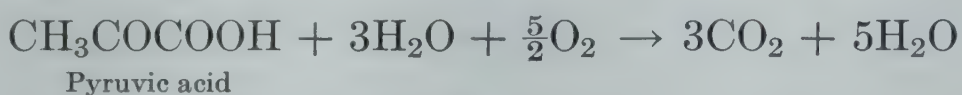




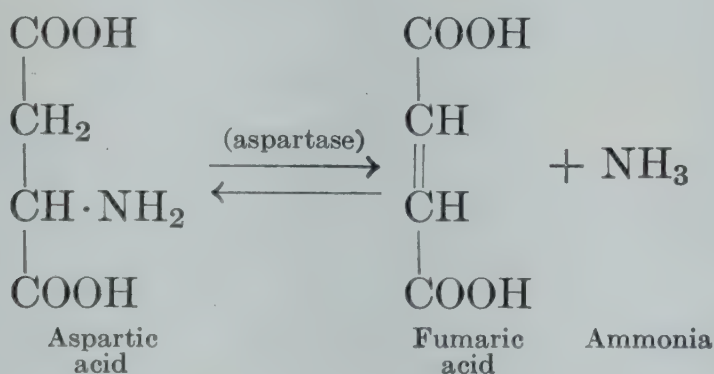
THE KREBS CITRIC ACID CYCLE

Although a few of the intermediate compounds of carbohydrate metabolism, such as glucose, glucose-6-phosphate, and glyceraldehyde phosphate, can be dehydrogenated, it is believed that the principal oxidations take place with a series of acid substrates which arise from pyruvic acid. These acids, shown in the diagram on p. 152, are called *acids of the Krebs cycle*. The enzymes which catalyze the dehydrogenation of several of these compounds have been studied in vitro as well as in vivo.

The net result of the transformations which take place at each turn of the cycle is the oxidation of a molecule of pyruvic acid with the formation of CO_2 and H_2O .

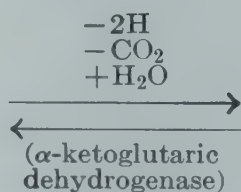
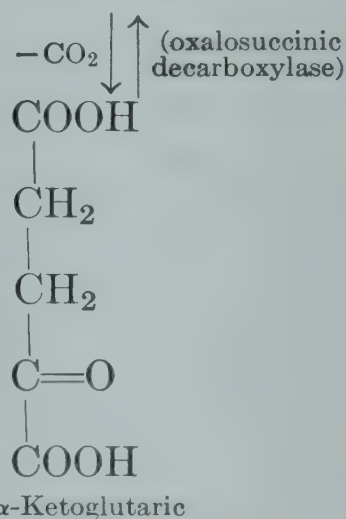
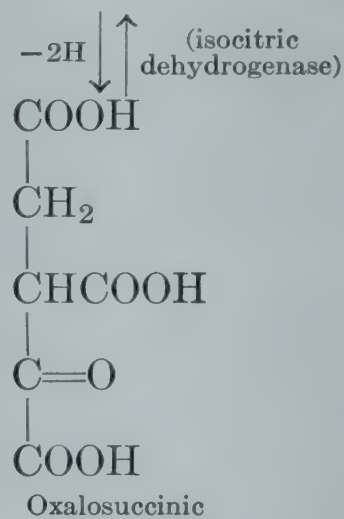
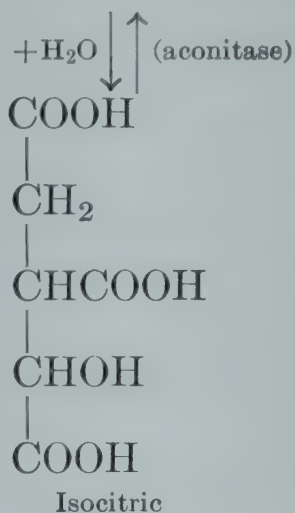
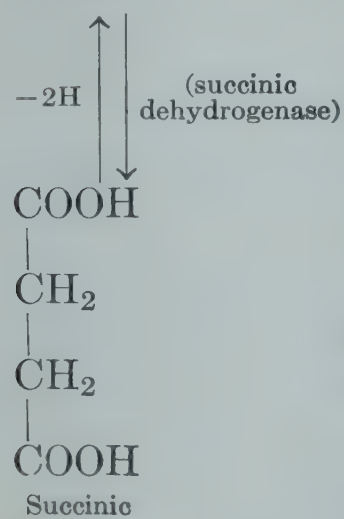
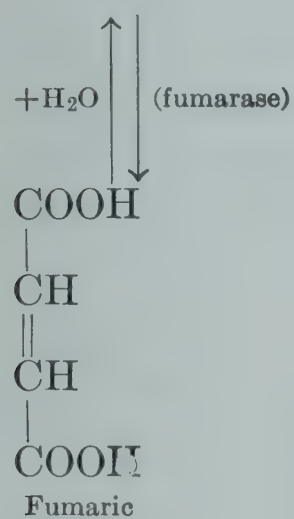
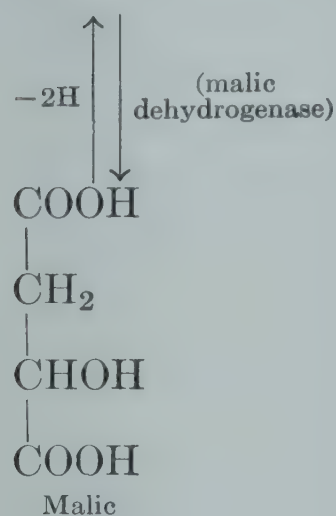
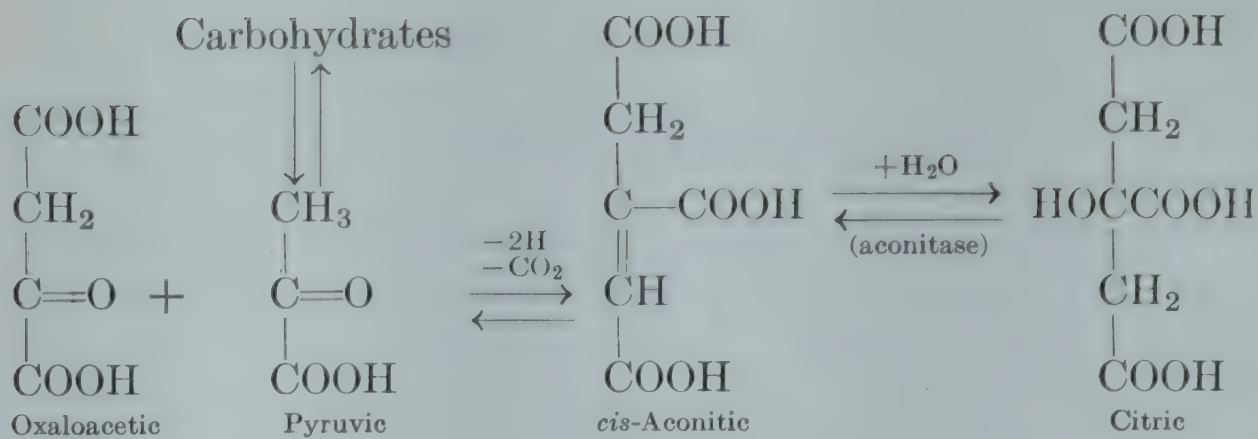


Protein metabolism and the Krebs cycle. Proteins are hydrolyzed to amino acids with the aid of the proteolytic enzymes which were discussed in the previous chapter. Amino acids can be deaminized by deaminases, amino acid oxidases, and transaminases. Thus alanine becomes pyruvic acid, glutamic acid is changed to α -ketoglutaric acid, and aspartic acid is deaminized to fumaric acid.



Hence protein metabolism also gives rise to products which are components of the Krebs cycle.

Fat metabolism and the Krebs cycle. Recent work has shown that oxidases exist which catalyze the oxidation of fatty acids. The immediate products of this oxidation are acetic acid and acetoacetic acid. One of these compounds or a derivative, such as acetyl phosphate, unites with oxaloacetic acid to form citric acid. Since cells can change citric acid to isocitric acid by the action of aconitase, it now seems likely that some of the

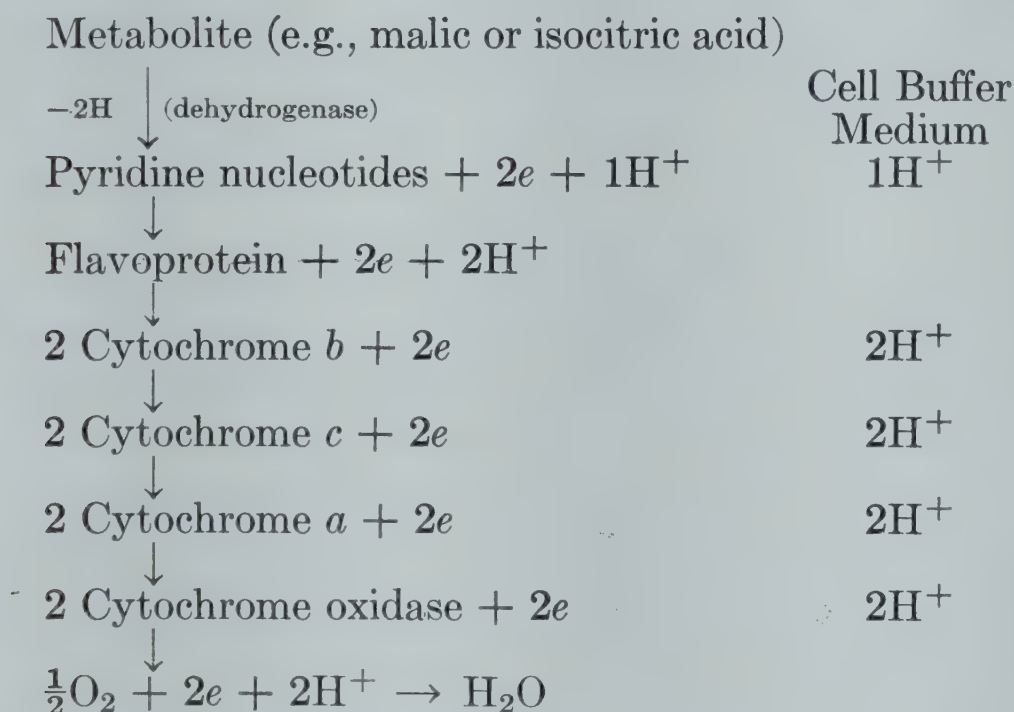


Krebs Citric Acid Cycle

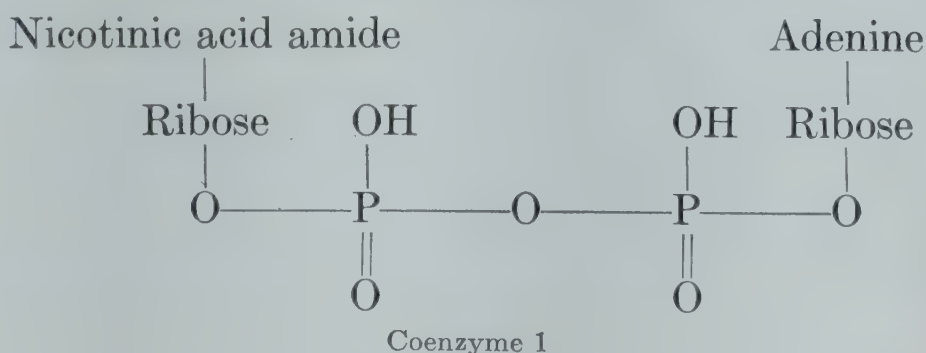
products of fat metabolism eventually become acids of the Krebs cycle.

TRANSFER OF ELECTRONS AND PROTONS

Dehydrogenases. The first step in the oxidation of a metabolite is the removal of $2H$ from the substrate by a dehydrogenase. Most of the dehydrogenases responsible for this action require coenzymes for their action. It is these coenzymes, the pyridine nucleotides, which act as the first carriers of electrons and protons. These agents are capable of being reversibly oxidized and reduced. After being reduced, by accepting electrons, they are in turn oxidized by passing these electrons to other compounds. Several such electron transfer agents or carriers are used to pass electrons from the substrate to oxygen. The protons (H ions) are carried by the cell buffer medium (see the following chart):



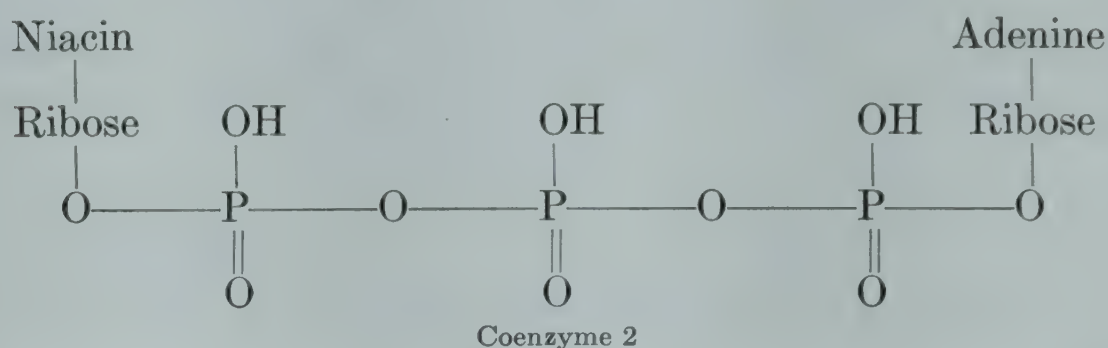
Coenzyme 1. Coenzyme 1 is composed of one molecule of nicotinic acid amide, one molecule of adenine, two molecules of *D*-ribose, and two molecules of phosphoric acid.



The compound is a diphosphopyridine nucleotide often abbreviated to DPN. Other names for coenzyme 1 are cozymase, co-dehydrogenase 1, and yeast coenzyme. The most reactive part of the molecule is the nicotinic acid amide residue which reversibly adds two electrons and one proton. Nicotinic acid amide, also called niacin, is a member of the vitamin B complex.

Coenzyme 1 acts as coenzyme for lactic dehydrogenase, ethyl alcohol dehydrogenase, malic dehydrogenase, glutamic dehydrogenase, and others. It is oxidized by passing its electrons to a flavoprotein.

Coenzyme 2. Coenzyme 2 contains one more phosphoric acid group than does coenzyme 1, but in other respects the two coenzymes are thought to be identical.



Coenzyme 2 is a triphosphopyridine nucleotide, often referred to as TPN. It has also been called Warburg's coferment and the "hydrogen-carrying coenzyme of red blood cells." Coenzyme 2 acts as the coenzyme for isocitric dehydrogenase, glucose-6-phosphate dehydrogenase, and a few other dehydrogenases.

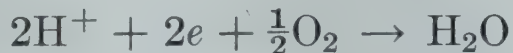
Both coenzyme 1 and coenzyme 2 undergo the same type of oxidation-reduction reaction. Both coenzymes transfer electrons to flavoproteins.

Flavoproteins. The flavoproteins, also called yellow enzymes, are enzymes containing riboflavin (vitamin B₂). The prosthetic group of these enzymes has been found to be riboflavin phosphate. Enzymes containing this vitamin derivative include Warburg and Christian's yellow enzyme, diaphorase, xanthine oxidase, D-amino acid oxidase, and L-amino acid oxidase.

In the system of electron transport the flavoproteins are oxidized by transferring two electrons to cytochrome. The protons are given off to the cell buffer medium.

Cytochromes. At least three different cytochromes are recognized. They are called cytochromes *a*, *b*, and *c*. That they are actually different has been ascertained from their absorption spectra. Each of these three cytochromes has as its functional group an iron atom. This iron atom is attached to four pyrrole nuclei in such a way that the valence of iron may be either two or three. In the oxidized state the Fe^{+++} accepts one electron and becomes Fe^{++} . This electron in turn can be passed on from cytochrome *b* to *c* to *a*. Cytochrome *a* passes the electron to cytochrome oxidase. As each cytochrome loses an electron, its iron is reoxidized to its original ferric form.

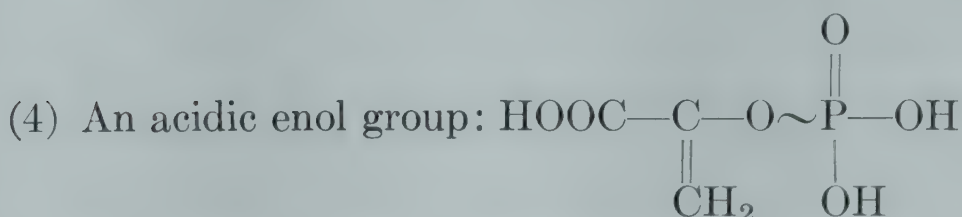
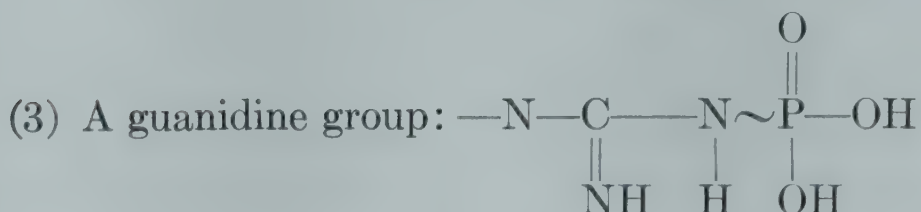
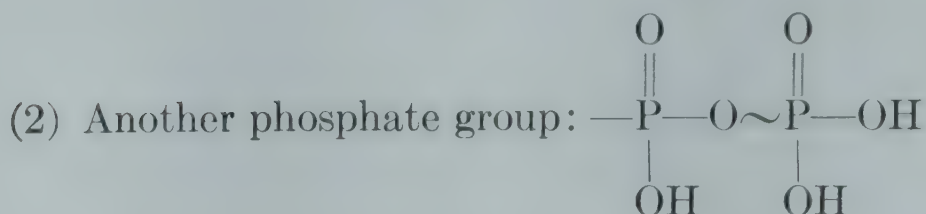
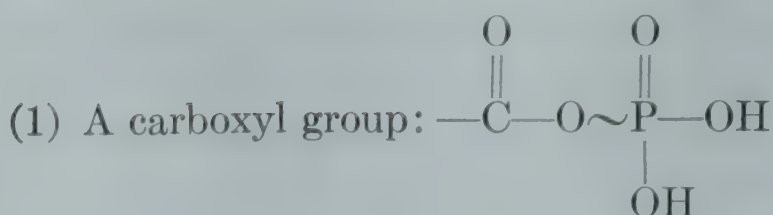
Cytochrome oxidase. Cytochrome oxidase is the final electron carrier in the chain which reaches from the substrate to water. This enzyme is the only catalyst of the chain that has the power to utilize molecular oxygen. Cytochrome oxidase contains iron which can be reversibly oxidized and reduced in the same manner as the cytochromes. This enzyme catalyzes the formation of water from (1) protons of the buffer medium, (2) electrons passed to it from cytochrome *a* and (3) atmospheric oxygen. The reaction catalyzed is:



Water is thus the graveyard of electrons from oxidized food.

Energy transfer in metabolism. The metabolism of carbohydrates has been shown to take place by small steps through a complex series of reactions. This series of reactions liberates energy for the use of living organisms. The one known mechanism for the utilization of energy is the formation and breaking of *high-energy phosphate bonds*. The term high-energy is applied to these bonds because, when broken, they release about 12,000 calories of energy per mole, whereas an ordinary phosphate ester bond releases about 2000 calories. In the latter type of low-energy bond the phosphate is linked through an alcoholic hydroxyl group. Examples of compounds in this group include hexose phosphates, triose phosphates, 2-phosphoglyceric acid, and 3-phosphoglyceric acid.

Four types of high-energy phosphate bonds are recognized, although others may exist. These bonds (shown as $\sim\text{P}$) are formed between phosphate and



As postulated in the Krebs cycle transformations, the oxidation of one molecule of pyruvic acid is accomplished by the removal of five pairs of hydrogen atoms from various intermediate compounds. Each pair of electrons, from these hydrogen pairs, gives up energy in the transfer from the Krebs cycle compounds to oxygen. This energy is used in the creation of high-energy phosphate bonds.

Examples of compounds containing high-energy phosphate bonds will be found in Chapter 20. Some of these compounds are D-1,3-diphosphoglyceric acid, adenosine di- and triphosphate, creatine phosphate, and enolphosphopyruvic acid.

OTHER OXIDIZING ENZYMES

There are a large number of known oxidizing enzymes which do not function as part of the connected series we have just discussed. Among these oxidizing catalysts are several examples of *copper-containing enzymes*, such as tyrosinase and ascorbic acid oxidase, the *amino acid oxidases*, and two *iron-containing enzymes*, catalase and peroxidase.

Tyrosinase. Tyrosinase catalyzes the oxidation of tyrosine, phenol, *m*-cresol, *p*-cresol, catechol, 3,4-dihydroxyphenylalanine (dopa), and other substrates. It does not attack *o*-cresol or hydroquinone. Tyrosinase requires oxygen for its action. The first step in the oxidation of tyrosine is the introduction of a second hydroxyl group in the *ortho* position. The resulting compound, 3,4-dihydroxyphenylalanine, is also acted upon by tyrosine and is changed to the corresponding quinone. This compound is believed to add water, forming the dark-colored pigment, *melanin*.

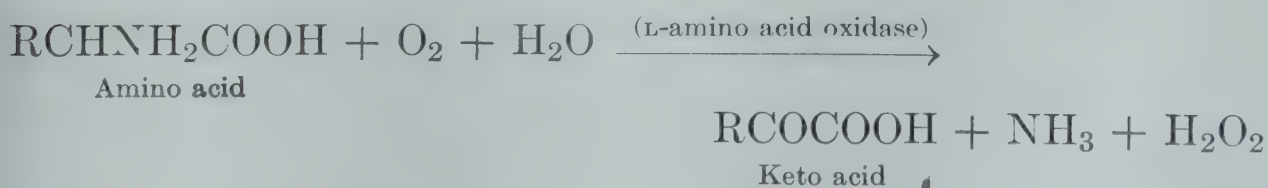
The meal worm and the potato are good sources of tyrosinase. It is found in many plants and is present in invertebrates. The darkening of sliced potatoes is due to the production of dark melanin pigments through the action of tyrosinase.

Ascorbic acid oxidase. The function of ascorbic acid and its oxidizing enzyme, ascorbic acid oxidase, is not well understood. The enzyme undoubtedly plays a role in biological oxidations, but its exact function has not yet been determined. Ascorbic acid oxidase catalyzes the oxidation of L-ascorbic acid to dehydro-ascorbic acid.

Ascorbic acid oxidase occurs in many plant products. It has been found in squash, cucumbers, bananas, lettuce, spinach, string beans, and other plants.

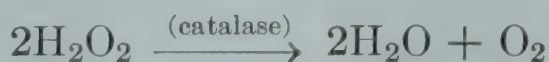
Amino acid oxidases. Oxidases that catalyze the oxidation of both D- and L-amino acids are known. Since naturally occurring amino acids are L acids, the presence of D-amino acid oxidase is somewhat unusual. It has been postulated that oxidases are present to remove the D-amino acids before they can be built into proteins.

L-amino acid oxidase, isolated from rat kidneys and livers, has been found to be a flavoprotein. This enzyme catalyzes the oxidation of thirteen L-amino acids.

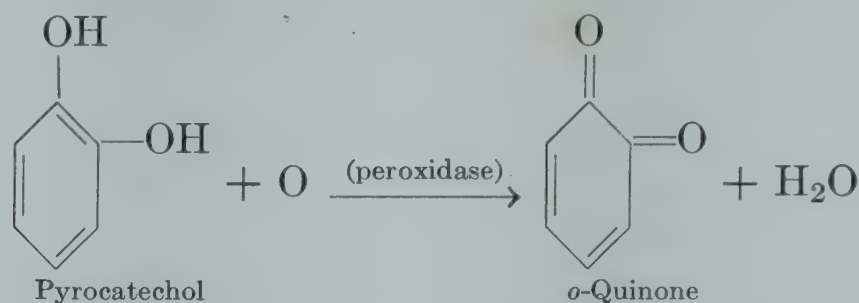


Catalase. The reaction in the previous paragraph illustrates the fact that H_2O_2 can be formed in living cells and, since an

appreciable concentration of H_2O_2 would prove toxic to cells, some means must be present to remove it. This is the function of catalase, an iron-containing enzyme, which catalyzes the reaction by which toxic hydrogen peroxide is changed rapidly to water and molecular oxygen. Catalase is found in more tissues than any other enzyme studied. It has been found in most cells but is lacking in anaerobic bacteria.



Peroxidase. Peroxidase is an iron-containing enzyme which catalyzes the oxidation of several substrates by means of oxygen liberated from H_2O_2 . The decomposition of the H_2O_2 by the enzyme seems to occur only in the presence of a suitable oxidizable substance. Such substrates include pyrocatechol, ascorbic acid, tyrosine, guaiacol, *p*-cresol, pyrogallol, and bilirubin. The coupled reactions catalyzed can be illustrated as follows:



It is generally believed that peroxidases are present in nearly all plant tissues. Horseradish is a particularly rich source of this enzyme. Peroxidase is probably not present in most animal organs, nor in anaerobic bacteria.

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Part 2 ·
The Plant

9 · Seed Germination

A mature seed contains an embryo and stored food enclosed in one or two seed coats. The *embryo* is the potential plant which develops from the fertilized egg while the seed is a part of the parent. The growth of this embryo is arrested in the mature seed, and the embryo remains in a state of rest as long as the seed is stored in a cool, dry place. The resumption of growth of the embryo after this dormant period is called *germination*.

Since the embryo cannot synthesize its own food, it must depend upon stored reserve materials for its nourishment. When a seed is placed in the proper environment, the embryo resumes its growth by utilizing the stored food within the seed coat and eventually becomes a more highly developed structure with some of the characteristics of the parent plant. At this stage of its growth the young plant is called a *seedling*.

Chemical composition of seeds. Seeds contain variable quantities of the elements and compounds that are necessary for the formation of new tissues. Organic compounds such as lipids, proteins, and carbohydrates predominate. Various elements, in addition to those found in the three large classes of compounds just mentioned, are also present. Both macro- and micronutrient elements are present in sufficient amounts to assure the growth of the young seedling to the point where it may secure these elements from soil or nutrient medium in which it is grown.

The composition of seeds produced by one plant species under similar environmental conditions is quite constant, although seeds of different varieties may be expected to differ slightly. Marked differences are found in the composition of seeds of different plant species.

Soybean is rich in protein; wheat, oats, corn, and barley con-

tain large amounts of starch; flaxseed and peanuts have high fat contents.

CHEMICAL COMPOSITION OF SEEDS

Kind of Seed	Water, %	Ash, %	Crude Protein, %	Crude Fiber, %	N-Free Extract, %	Lipids, %
Barley	10.6	2.8	12.7	5.4	66.6	1.9
Corn, dent No. 1	13.0	1.2	8.8	2.1	70.9	4.0
Cottonseed	9.4	4.6	19.5	22.6	24.9	19.0
Flaxseed	6.2	3.6	24.0	6.3	24.0	35.9
Oats	9.8	4.0	12.0	11.0	58.8	4.6
Peanut kernels	5.4	2.3	30.4	2.5	11.7	47.7
Soybeans	10.0	4.6	37.9	5.0	24.5	18.0
Wheat	10.5	1.9	13.2	2.6	69.9	1.9

FACTORS INFLUENCING THE PROCESS OF SEED GERMINATION

Certain conditions are necessary for the germination of a seed. Adequate moisture, sufficient oxygen, and a suitable temperature must be provided. The age of the seed, its previous treatment, and light are also important factors affecting germination.

Moisture. The water content of stored seeds of the common agricultural plants is usually about 5 to 12 per cent. This moisture content is too low to allow rapid metabolism, and the first step in the germination of these seeds must be an increase of the water content.

The compounds present in embryos, storage organs, and many seed coats have a marked affinity for water. Some seeds have such a strong attraction for water that they can secure sufficient water for germination from an air-dry soil. Clover and alfalfa seeds, under favorable conditions, absorb quantities of water equal to their original weight; other seeds such as wheat and corn absorb water equal to about one-half their weight. The imbibition of water produces a large increase in pressure within the seed, the contents swell, and the coat may be ruptured.

The affinity of most seeds for water is insufficient to secure adequate moisture for germination from solutions of high osmotic pressure. For this reason high concentrations of soluble fertilizers should not be placed close to germinating seeds.

Some seeds such as red clover possess hard impervious coats which do not allow water to pass. These seeds are frequently prepared for germination by treatment with sulfuric acid, or they are blown against needle points which scratch or puncture the outer coat. Treatment with ZnCl_2 in HCl (Cross and Bevan's solution), extraction with fat solvents, and many other methods have been used to remove obstacles to the penetration of water.

Seeds respire during storage even though this process takes place at a low rate. One of the factors which determines the rate of this respiration is the moisture content of the resting seed. If seeds are stored under conditions of relatively high humidity their vitality may be seriously impaired. The critical moisture content of stored seed depends on the temperature. As the temperature is lowered the moisture content can be increased without harmful effects.

Oxygen. Seeds have characteristic requirements as to the amount of oxygen needed for germination. Most seeds germinate in the presence of air, whereas others, such as those of cattails, germinate poorly or not at all unless the oxygen supply is reduced. As a rule, any condition leading to a lack of oxygen and an accumulation of carbon dioxide is harmful to germination. In some cases the germination process is hindered by the presence of a few hundredths of a part of carbon dioxide in one part of oxygen. A thick covering of compact soil may bring about such a condition. The thickness of the soil cover which is best for germination depends upon the physical condition and the moisture content of the soil as well as the kind of seed. An interesting example of delayed germination due to a lack of oxygen is found in the cocklebur. Each cocklebur contains two seeds; the lower of the two germinates the first year after ripening, whereas the upper seed may germinate a year or more later. A thin membrane surrounding the upper seed prevents sufficient oxygen from reaching the embryo and results in delayed germination. This membrane must be made more permeable to oxygen before germination will take place. Freezing, decay, or high temperatures will accomplish this. Other seeds are also known that lie dormant in the soil due to lack of oxygen, for, although they absorb moisture readily, they possess membranes which reduce the oxygen supply to the embryo. Viable seeds respire

during storage, but such respiration is at a very low rate, for there are records of seeds that were still alive after many years of storage. Thus the seeds of *Nelumbium* (a species of large water lilies) were alive after storage for 250 years. Since the respiration rate of such long-lived seeds is very low, only minute amounts of oxygen are required during their storage.

Temperature. The optimal temperature range for the germination of seeds of most agricultural plants seems to be from 68 to 86° F. Some seeds such as those of celery, bluegrass, and redtop germinate better under certain alterations of temperature than at constant temperatures. Most seeds fail to germinate at temperatures as low as 32° F, but a period of moist storage at temperatures of 35 to 55° F results in better germination of many seeds. Low-temperature treatment of seeds often results in a more rapid and vigorous growth of the seedlings. Although the effect of such low-temperature treatment is well known, the reason why a seed so treated will later germinate more rapidly and grow better is not known. Temperatures as high as 110° F prevent the germination of most seeds.

Age of the seed. Seeds degenerate with age, although a certain percentage of various seeds will germinate after storage for many years. The reason for this loss of vitality is probably not the loss of stored foods, for even aged seeds contain large amounts of reserve food material. However, changes in the protein fraction of seeds have been found to take place. A decrease in true protein content and an increase in smaller compounds such as amines, amides, and amino acids have been shown to occur. This has been interpreted as indicating that longevity of seeds depends on the retention of the original structure of their proteins. As soon as too many of the original proteins have degenerated it is no longer possible for the seed to germinate because it cannot form the new nitrogen compounds, particularly proteins, essential for the development of the embryo. This theory is unproved but at least has some grounds for credibility. If protein degeneration results in the production of compounds from which the proteins of enzymes can no longer be formed, the seed will not germinate.

Many seeds require a period of aging before they will germinate properly. This aging period has been called a *rest period* or a

period of *after ripening*. Freshly harvested seeds may contain inhibiting substances that volatilize or decompose during dry storage. Such inhibitors are known to be present in the coats of fresh lettuce seeds, in the woody materials of the seed balls of beets, and in the fleshy fruits of tomato and cucumber. The fact that seeds will not germinate while in the fruit but retain their ability to germinate after the fruit covering has been removed is explained by the presence of inhibitors. These chemical inhibitors include such substances as ammonia, hydrocyanic acid, essential oils, alkaloids, and glycosides.

Seed treatment. Treatment of seeds in order to control certain plant diseases, particularly diseases caused by fungi, has been practiced for many years. Copper sulfate was used as a seed fungicide in 1761. Other copper compounds which were introduced subsequently include the carbonate, oxide, chloride, oxychloride, and arsenate. Copper compounds have been applied for the control of such diseases as bunt and stinking smut of wheat.

Organic and inorganic compounds of mercury have been used to control seed diseases. Mercuric chloride, for example, has been used extensively for the treatment of seed potatoes to prevent scab and for the treatment of wheat to control the *Fusarium* organism. Organic mercurials have been used to treat corn, peas, wheat, oats, and other seeds. Various commercial preparations such as Uspulum, Semesan, and Ceresan are available in Europe and the United States. Ceresan, which contains ethyl mercuric chloride, is recommended for treating cotton, pea, and flax seeds.

Other fungicides used for seed treatment include salts of zinc, nickel, and lead, and the elements sulfur, iodine, and chlorine gas. The chemistry of these compounds will be discussed in a later chapter. Formaldehyde, paraformaldehyde, chloranil, phenols, and other organic compounds have also been used.

A hot-water treatment introduced in 1887 is quite effective in controlling the loose smuts of wheat and barley. One of the methods of this treatment consists in placing presoaked seeds in water held at 54° C for 10 minutes. Tests on wheat show that there is a 20 per cent reduction in the number of seeds which germinate after hot-water immersion. The margin of safety in this treatment is quite narrow. When either time

or temperature is increased much above 10 minutes and 54° C, seed enzymes are affected and all the seeds may be killed.

In general, fungicides have an inhibitory effect on enzyme action, and most of the fungicidal agents exert an unfavorable effect on seed germination. It is usually believed that a toxic substance must enter tissues in order to be toxic to an organism. There is evidence that several fungicides penetrate seeds. Thus it has been found that potatoes sprayed with Bordeaux mixture contained more copper within the tuber than untreated potatoes. It has been noted that seeds with damaged seed coats are more likely to be killed or inhibited after treatment than the same variety with intact seed coats.

In most cases fungicidal treatment of seeds seems to increase the time needed for complete germination. The yields of agricultural crops from treated seed are often depressed unless the seeding rate is increased. It has been reported that certain seed treatments stimulate the germination process. But in most cases the benefits derived from seed treatment are due to control of organisms found either on the seed or in the seed bed, and not to stimulation of germination.

Light. Germination of most seeds is favored by light, although light inhibits the germination of a few seeds such as members of the pigweed family. The seeds of several common agricultural plants such as corn, small grains, clover, and beans germinate equally well in the presence or absence of light.

METABOLISM OF GERMINATING SEEDS

When seeds are placed in an environment favorable to germination, the slow metabolism of the resting seeds becomes rapid and intense. The reactions taking place include hydrolysis, oxidations, desmolyses, and syntheses. Stored food is changed from insoluble, immovable substances to soluble, transportable compounds which are translocated to the embryo. With these soluble compounds as building blocks, the embryo can synthesize compounds needed for the manufacture of new tissue. There is a great increase in enzyme activity of seeds during germination. Carbohydases, proteinases, aminases, lipases, oxidases, and desmolases are active in germinating seeds.

Carbohydrate metabolism. The main storage carbohydrate of seeds is starch. The amylose and amylopectin components of starch are broken down by amylases or by phosphorylase. The result of the hydrolysis of starch by amylases is a mixture of glucose, maltose, and small dextrins. Maltase will hydrolyze the maltose to glucose. Low molecular weight dextrins are also hydrolyzed to glucose, but the method by which this is accomplished is uncertain. Glucose produced by amylase and maltase action is converted to glucose-6-phosphate by hexokinase.

Peas, beans, and potatoes are rich in phosphorylase. Most seeds probably contain this starch-splitting and starch-forming enzyme. Phosphorylase converts starch to glucose-1-phosphate. Glucose-1-phosphate is changed to glucose-6-phosphate by phosphoglucomutase. Thus starch hydrolysis, by means of amylases, maltase, and hexokinase or by phosphorylase and phosphoglucomutase, results in the formation of the same compound, glucose-6-phosphate.

Glucose-6-phosphate is transformed by a series of changes which were described in our discussion of intermediary carbohydrate metabolism. This involved the splitting of a hexose to two trioses and the oxidation of the trioses to pyruvic acid. Pyruvic acid may be oxidized to carbon dioxide and water through a series of transformations in the Krebs acid cycle. The intermediary compounds and the Krebs cycle acids can be used for the synthesis of fats, proteins, or new carbohydrates required for the growth of the embryo and seedling.

Some germinating seeds, e.g., barley, contain large amounts of sucrose. It is now known that sucrose can be formed by the reaction:

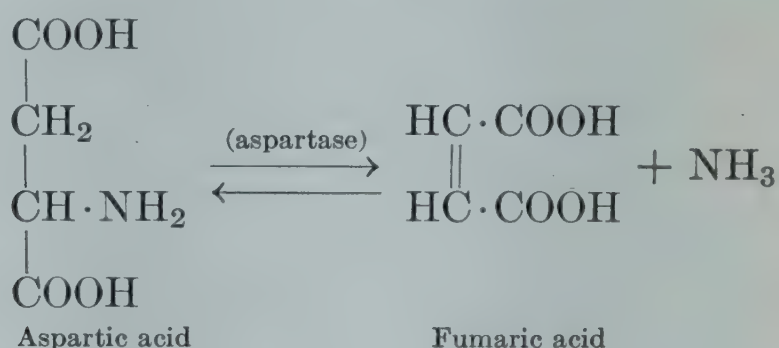


The enzyme that catalyzes this reversible reaction is called *sucrose phosphorylase*. So far, it has been found only in certain bacteria. Whether or not this is the reaction that takes place in germinating seeds, it is probable that fructose or a fructose phosphate is necessary for the formation of sucrose. Fructose phosphates are produced in the breakdown of starch to pyruvic acid. Fructose is produced by the hydrolysis of fructose-6-phosphate by a phosphatase. Sucrose in seeds can be hydrolyzed

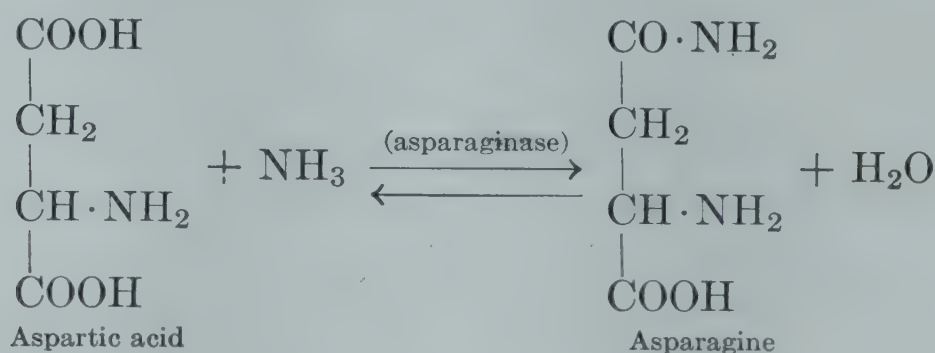
to glucose and fructose by the action of sucrase. This reaction has been discussed in the previous chapter on enzymes.

Protein metabolism. Protein reserves of the seed are rapidly changed into simpler compounds during germination, and these are translocated to the point of utilization. Protein breakdown is initiated by proteinases of seeds. These plant enzymes belong to the papainase group which are inactive when in the oxidized form but are activated by reducing agents. Papainases hydrolyze proteins to a mixture of polypeptides and small amounts of amino acids. The polypeptides are then hydrolyzed to amino acids through the action of peptidases.

The amino acids may be utilized directly for the production of essential plant compounds such as enzymes and nucleoproteins, or they may be utilized indirectly through the formation of other compounds. Amino acids can be deaminized by deaminases (aminases), amino acid oxidases, or transaminases. An illustration of the deamination of an amino acid by deaminases of seeds is the action of aspartase.



The ammonia produced can react with organic acids to form amides. When ammonia reacts with a molecule of aspartic acid in the presence of asparaginase it forms *asparagine*.



Another well-known reaction is the addition of ammonia to glutamic acid to form *glutamine* in the presence of glutaminase.

Asparagine and glutamine are excellent carriers of nitrogen for protein synthesis. These compounds can be broken down to give products available for the synthesis of the particular amino acids and proteins required for growth of the embryo.

Amino acid oxidases produce ammonia, hydrogen peroxide, and keto acids from amino acids. Keto acids also result when amino acids lose their amino groups by transamination. The keto acids can be decarboxylated, forming the corresponding aldehydes, or they may be utilized through the pathway of the Krebs cycle transformations.

Fat metabolism. The fats and oils of mature seeds are found in relatively large globules. During the resting period and during germination these globules are reduced in size, thus providing more surface for the action of fat-splitting enzymes.

In the presence of sufficient water, the lipases of seeds rapidly hydrolyze the fats and oils to glycerol and fatty acids. Neither the fatty acids nor the glycerol accumulate in large concentrations during germination but are quickly changed to other compounds. The principal products formed from fats during the germination have been shown to be carbohydrates.

A suggested mechanism for the conversion of fats into carbohydrates is as follows: (1) Fats and oils are hydrolyzed by lipases to fatty acids and glycerol. (2) Fatty acids are oxidized by fatty acid oxidases to acetic acid and acetoacetic acid. (3) One of these compounds or a derivative reacts with oxaloacetic acid to form citric acid. (4) Citric acid is changed to aconitic acid by aconitase. (5) Aconitic acid, found in the Krebs cycle, will form carbohydrates by the reactions of carbohydrate metabolism, as shown in the previous chapter. (6) Glycerol is converted to α -glycerophosphate. (7) The α -glycerophosphate is dehydrogenated to a triose phosphate. (8) Triose phosphates combine to form hexose phosphates.

10 • The Soil and Its Relation to Plant Growth

As the seedling develops, it acquires the ability to synthesize the organic compounds necessary for its growth. It is independent of further supplies of organic matter after it has exhausted the available food originally contained in the seed. The young plant now requires a supply of inorganic materials.

The elements necessary for plant growth include carbon, hydrogen, oxygen, phosphorus, potassium, nitrogen, sulfur, calcium, iron, magnesium, boron, molybdenum, manganese, copper, and zinc. Several other elements, if not essential for all, are beneficial to a number of plants. Such elements include aluminum, sodium, chlorine, and silicon.

The plant secures oxygen and carbon from the surrounding air, but the soil supplies most of the elements necessary for or beneficial to plant growth. The absorption and utilization of these elements by plants depends on the operation of a number of factors discussed in this chapter.

THE SOIL

Soil is the loose, easily crumbled part of the earth's crust in which plants find support and nourishment. This loose, friable material is a mixture of four major components: inorganic material, organic matter, water, and air. The process by which this mixture is formed and becomes a suitable medium for plant growth is complex.

The inorganic material is usually present in greatest amount and has originated principally from rocks and decomposition products of rocks. These inorganic substances vary in size from coarse particles, such as rocks and gravel, to very fine particles, such as silt and clay.

A detailed consideration of soil formation, an important branch of soil science, is beyond the scope of this textbook. However, the principal forces involved in the formation of soil are: (1) physical forces causing disintegration and mechanical subdivision of rocks and rock particles, (2) chemical forces causing decomposition, and (3) biological forces resulting in both disintegration and decomposition.

Physical forces. The physical forces acting on parent rock and smaller particles include: *changes of temperature*, causing expansion and contraction; *freezing of water* in cracks and crevices; *abrasion* when boulders, rocks, gravel, and sand are carried by streams of water; *wind-borne sand*, which grinds away native rock and moves particles to new locations; *glaciers*, which pick up rock and rock particles and cut, grind, and crush native rock over which they move.

Chemical forces. Simple *solution* is probably the first reaction which takes place between water and any kind of rock mineral. Among the materials most readily dissolved are compounds of sodium, potassium, calcium, and magnesium. When water dissolves a mineral such as calcium silicate, *hydrolysis* takes place with the formation of calcium hydroxide and silicic acid.



The silicic acid may lose water and deposit SiO_2 , a very unreactive compound. The calcium hydroxide may be washed away as such, but it is more likely to be changed by *carbonation* to calcium carbonate. Carbonic acid, the carbonating agent, may arise from the carbon dioxide produced by plant roots, by decomposing organic matter, or by microorganisms, from carbon dioxide found in air, or from carbonates of rocks and minerals. Carbonation affects compounds containing sodium, calcium, magnesium, potassium, and ferrous iron.

Hydration plays an important part in chemical decomposition by forming hydrated products of minerals. A large number of

silicates, oxides, and carbonates will form hydrated compounds. These compounds are more reactive than the original complexes.

Most of the minerals found in rocks are already fully oxidized. Exceptions include minerals containing ferrous iron, e.g., pyrites, which can be changed to ferric compounds by *oxidation*. This process requires oxygen and water and takes place more rapidly in humid than in dry regions. Oxidation of elements such as sulfur and nitrogen leads to the formation of mineral acids which have a solvent action on rock constituents.



Biological forces. Rocks may be broken by the expansion of plant roots in cracks. Mosses and lichens produce disintegration when they grow in intimate contact with rock and mineral surfaces. Various microorganisms are active in the decomposition of parent soil constituents. Animals, such as rodents and earthworms, aid in mixing various horizons of soil and in supplying fresh subsoil to surface layers.

When sufficient soil has been created to supply a substrate for higher plants, soil formation is accelerated. The roots of these plants contribute carbon dioxide, and, when they die, organic matter which in turn acts as substrate for microorganisms. Products of microorganism metabolism, including carbon dioxide, ammonia, and organic acids, hasten the decomposition of rock material. The intimate relationship between the resulting soil, the plant world, and the animal world is an essential feature of life on this planet.

INORGANIC MATTER IN SOILS

Most of the chemical elements known to man may be expected to exist in the soil, and a large number of them have been found to be present. However, the greater part of the inorganic matter in soil is composed of relatively few elements.

The approximate average composition of soils suitable for agriculture is as follows:

ELEMENT	OXIDE	PERCENT-
		AGE
Silicon	SiO_2	78
Aluminum	Al_2O_3	10
Iron	Fe_2O_3	5
Potassium	K_2O	2
Calcium	CaO	1
Magnesium	MgO	1
Sodium	Na_2O	1
Titanium	TiO_2	1
Others		1
		<hr/> 100

The composition of crop-producing soils may vary widely from the average given above. Thus American agricultural soils have been found to contain as low as 44 per cent and as high as 97 per cent silica. Aluminum oxide has been found to vary from below 2 per cent to 27 per cent. All soils contain small quantities of many elements not listed above, including manganese, phosphorus, and sulfur.

The composition of soils is usually expressed in terms of the oxides of the elements. However, the assumption should not be made that all elements exist as their oxides in soil. The only common oxide present in large amounts is the oxide of silicon, SiO_2 . Most elements are present as parts of complex compounds such as iron or aluminum silicates.

A study of soils and their composition has shown that there are four main groups of constituents important in determining the nature of a soil. The predominance of one or more of these four constituents will establish certain characteristics of a soil. Organic matter is one important constituent; the other three are groups of inorganic compounds:

1. Compounds of silicon, usually called silica.
2. Compounds of alkali and alkaline-earth metals. The important elements are Na, K, Mg, and Ca.
3. Compounds of iron and aluminum, referred to as the sesquioxides of iron and aluminum; e.g., Fe_2O_3 and Al_2O_3 .

Soils high in silica but low in the other two groups of inorganic constituents are usually acid. Such acid soils occur in cool,

humid climates and are generally open-textured, sandy, gravely, or stony. These cold-climate soils may contain large amounts of organic matter, often only semidecomposed. The soils of cold and moist climates called "gray earths," "forest soils," or "podzols" belong in the high-silica group.

Arid climates, whether hot or cold, allow the accumulation of alkali and alkaline-earth compounds in soils. If evaporation of water equals or exceeds rainfall there is little percolation of water through the soils and very little leaching of soluble compounds. Under these conditions alkali and alkaline-earth metals accumulate as carbonates and other basic compounds. As a rule such alkaline soils are low in organic matter. Irrigation of many arid-climate soils results in the production of crops with very large yields.

Under cool semiarid conditions organic matter and calcium accumulate, but the alkali metals, sodium and potassium, are not present in large amounts as basic compounds. Such soils, found in the wheat-growing area of western United States and Canada, are among the most fertile soils in the world. The black prairie grassland soils belong to this group.

A balanced distribution of organic matter, sesquioxides, alkaline-earth compounds, and silica is found in humid climate soils of central Europe and central eastern United States. Soils of these regions have a tendency to be slightly acid.

South temperate, subtropical, and tropical soils in humid climates are high in iron and aluminum. They contain less organic matter and less silica than soils in cooler climates, and they are usually low in calcium and magnesium. In such soils, called *laterites*, compounds of iron and aluminum are dominant. In moist tropical climates organic matter decomposes rapidly in the soil, producing CO_2 . The carbonates which are formed attack silica, forming more soluble compounds which are removed by leaching. Since alkali and alkaline-earth compounds also are rapidly lost, the predominant compounds become the iron and aluminum group.

SOIL ORGANIC MATTER

Soil organic matter is composed of the bodies of dead organisms and the excretions of living organisms deposited on or in the soil. Theoretically soil organic matter consists of only the non-living remains of organisms and the products of their decomposition. It is impossible to separate such material from the living microorganisms that inhabit the soil and are the most important agents in the decomposition of dead material. The bodies of living microorganisms are therefore commonly included as part of the soil organic matter. Thus our soil organic matter consists of dead roots, leaves, fruits, and stems of plants; carcasses of worms, insects, and animals; bacteria, fungi, and protozoa; and decomposition products of dead organisms.

Since the soil organic matter is a mixture of plant and animal material the chemical compounds present include carbohydrates, proteins, lipids, and other compounds found in plants and animals. The gradual decomposition of these compounds ultimately leads to the formation of such products as water, carbon dioxide, ammonia, methane, and simple inorganic salts. But before these simple end products are formed many intermediate compounds have been produced, some of them of less complexity than the original compounds, others of greater complexity.

Humus. The mixture of compounds resistant to decay formed during the decomposition of organic compounds in the soil is called humus. Soil organic matter is called humus when decomposition has proceeded to the stage where the specific structure of the original organized tissue has disappeared. Humus is characterized as an amorphous, dark-colored, nearly odorless material, having no definite chemical composition.

The carbon content of humus, about 55 to 58 per cent, is greater than the carbon content of the original plant and animal matter from which it is formed. The nitrogen content of humus is variable, usually falling in the range of 3 to 6 per cent. The ratio of carbon to nitrogen is therefore between 20 to 1 and 10 to 1, depending on the kind of organic matter from which it was derived, the stage of decomposition, the nature and depth of the soil, and the climatic conditions under which it was formed.

Since humus originates from the decomposition of plant, animal, and microbial residues, we can expect to find compounds in it which are similar to, or derived from, the compounds of the parent material. Thus, we find significant amounts of the following four groups of compounds: (1) Lipids and related materials. Fats, waxes, higher alcohols, resins, sterols, organic acids, and pigments are found in rather small amounts. (2) Carbohydrates and their derivatives. Polysaccharides such as cellulose, pentosans, hexosans, polyuronides, glycosides, starch, and sugars are present in varying amounts. (3) Proteins and other nitrogen compounds. This group of compounds, which includes proteins, proteoses, peptones, amino acids, amines, purines, and pyrimidine bases, is present in fairly large amounts. (4) Lignin and its derivatives. Lignin is one of the most stable compounds found in plants. It constitutes 25 to 30 per cent of the wood of trees and 15 to 20 per cent of straw. Lignin is present in large amounts in humus. The two major groups of compounds found in humus are thus the proteins and lignin. Probably most of the humus found in soils results from a combination of lignin with bacterial proteins and is commonly known as a lignoprotein.

Humus is nearly insoluble in water, but a part of it is soluble in dilute alkali solutions. Many of the important properties of humus depend on the fact that, alone or with inorganic substances of the soil, it forms complex colloidal systems. The properties and importance of soil colloids will be discussed later.

Two important groups of humus substances have been recognized: (1) *nutrient* or *temporary humus*, which serves microorganisms as a source of carbon and is easily broken down; (2) *persistent* or *maintenance humus*, which is slowly converted to other substances and forms the important organic colloidal material of soil.

Importance of soil organic matter. Organic matter has several important functions in soils: (1) It serves as a substrate for the growth of microorganisms. (2) It improves the physical condition of the soil by improving texture, moisture-holding capacity, and aeration. (3) It increases the buffering capacity of soil. (4) It combines with inorganic soil constituents to prevent their

loss by leaching but releases these substances for the use of plants. (5) It is the sole storehouse of nitrogen.

The importance of humus for the maintenance of soil productivity has been recognized for many years. Repeated observations have shown that virgin soils containing large amounts of organic matter in the form of humus may yield large crops for the first few years of cultivation. These crop yields gradually decrease with the number of years of cultivation and crop removal. One of the chief reasons for this loss of fertility is the loss of humus from the soil under continuous cropping.

However, the reader should remember that neither humus nor organic matter of any kind is essential to plant growth. Plants can be grown in sand cultures with properly balanced nutrient solutions containing no organic compounds, and plants can be grown in water solutions containing only inorganic compounds. The principal importance of soil organic matter, or humus, is its effect on the soil as a medium for plant growth.

SOIL COLLOIDS

Soil particles are classified arbitrarily according to size. An easily remembered classification is that made by the International Society of Soil Science.

SEPARATE	DIAMETER LIMITS, MM
Coarse sand	2.00–0.20
Fine sand	0.20–0.02
Silt	0.02–0.002
Clay	Below 0.002

The most important of the four groups from the standpoint of soil chemistry as related to plant growth is the clay fraction. It is this fraction which contains the soil colloids.

One of the most interesting properties of a fertile soil is its ability to hold plant nutrients with a force strong enough to prevent rapid loss of these nutrients by leaching, yet weak enough to enable plants to remove them. This property is largely dependent on the presence and properties of soil colloids. Colloidal particles also have an important influence on the physical structure, the water-holding capacity, and the buffer

action of soils. Since these factors play an important part in determining the fertility of a soil, the properties of the colloidal fraction must be related to soil fertility. Soil colloids are composed of both organic and inorganic materials.

Organic colloids. Colloidal humus is negatively charged and highly hydrated. It usually exists in the soil in the form of a gel which is mixed with, and coats, inorganic particles. The combined effects of its charge and hydration make it quite stable. As in the case of inorganic colloids, it will adsorb positive ions, which can function in base exchange.

Inorganic colloids. The inorganic fraction of soil colloids is composed principally of oxides of silicon, aluminum, and iron, with variable amounts of other basic elements. Since colloidal particles are usually considered to be less than 100 millimicrons in diameter, such particles will be found only in the clay fraction of soils. Particles with diameters less than 0.002 millimeter (2000 millimicrons) are classified as clay. Inorganic soil colloids are thus referred to as clay colloids, although not all the clay fraction is of colloidal size.

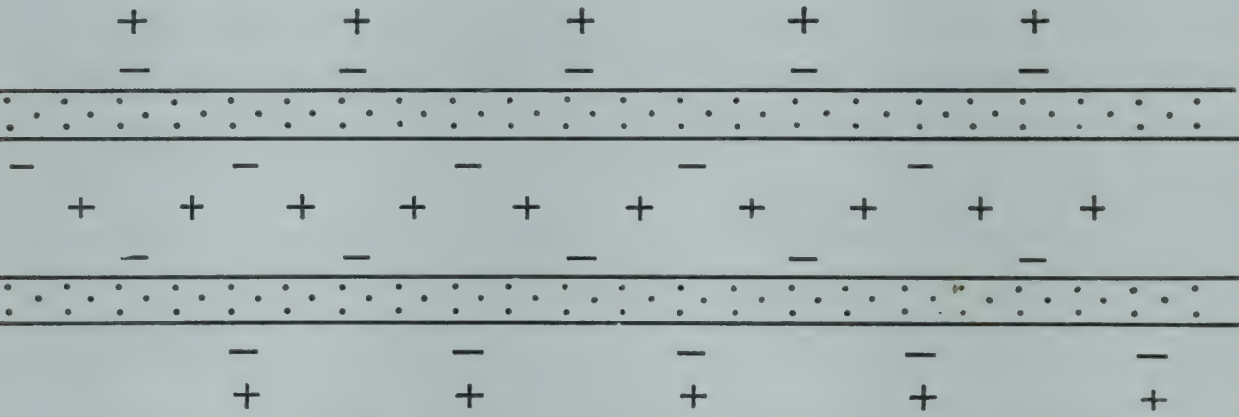


FIG. 9. Charged surfaces of two lamellas of a clay particle.

We have spoken of the diameter of clay colloids, but this does not mean that the colloids are spherical. Actually they are composed of laminated plates. That is, these particles are built up of several infinitely thin sheets of crystalline minerals. Such particles are present in a wide variety of shapes and sizes. Let us assume that a clay particle composed of at least two lamellas can be represented by the accompanying diagram. Because of its fine size and laminated structure this clay particle contains

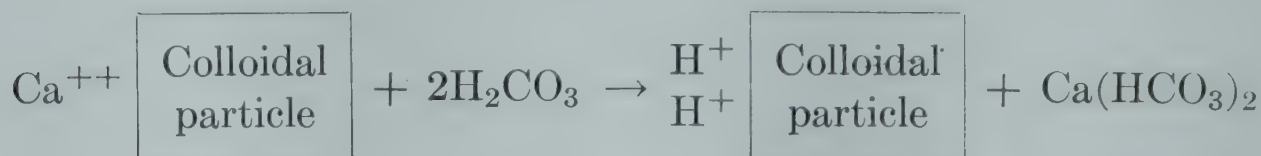
a large amount of external and internal surface. This surface is electrically charged and shows a definite electrical potential. The potential (zeta potential) is due to the presence of a double layer of ions called the Helmholtz double layer. The inner layer of ions is a fixed, negatively charged coat which forms an integral part of both the internal and external surfaces of the clay particle. It is the charge of this inner layer of anions which determines the characteristic charge of the colloidal micelle. Since this fixed inner layer has a negative charge in fertile soil, these soil colloids are negative and will migrate to the positive pole under the influence of an electrical current.

The outer layer of ions consists of positive ions which are readily exchanged for other cations. Calcium ions and hydrogen ions are present in greatest number, but potassium, magnesium, sodium, and other ions are also present. All these cations are hydrated, the number of molecules of water associated with each being a characteristic of the ion. Na^+ and K^+ carry a large number of water molecules; Ca^{++} and Mg^{++} carry less. The H^+ ions also carry water as they do at all times, and in newer chemical nomenclature they are properly called hydronium ions $(\text{H}_3\text{O})^+$. In addition to water of hydration carried by ions, water molecules are carried on the faces of these particles and by the spaces and channels between the thin sheets which make up the colloidal plates.

The composition of the clay colloidal material varies greatly and depends upon such climatic conditions as temperature and rainfall during their formation, as well as on the composition of the parent rock. The ratio of silica to sesquioxides in soil colloids varies and seems to have an important effect on the properties of these particles. A soil containing a high ratio of silica to sesquioxides shows greater negative charge, dispersibility, viscosity, swelling, heat of wetting, adsorption of bases, and rate of base exchange. These colloids retain their negative charge in both acid and alkaline solutions. If a soil has a low silica-sesquioxide ratio the mineral colloids show an amphoteric behavior, for they become electropositive in acid solutions and will then adsorb anions such as chloride ions and sulfate ions instead of positive ions.

BASE EXCHANGE

The fixation of a positive ion by a colloidal particle of the soil is accompanied by the release of one or more previously held positive ions. Such an exchange of ions is commonly called *base exchange* or, more properly, *cation exchange*. An illustration of cation exchange is found in the gradual acidification of soil through the decomposition of organic matter and subsequent leaching of calcium. As organic matter decomposes in the soil, carbon dioxide is produced and combines with the soil water to form carbonic acid. The hydrogen ion of this acid will replace positive ions in the outer layer of the colloidal soil particles. If the particles contain a large amount of adsorbed calcium, the following change takes place:



Since calcium bicarbonate is soluble in water, leaching will remove this compound from the topsoil. The loss of calcium in this manner will gradually increase the acidity of the soil.

The following principles have been well established for base-exchange reactions of soils:

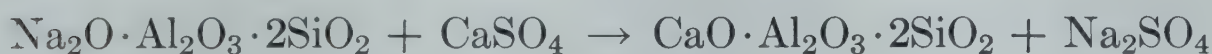
1. Adsorbed ions are replaced by other ions in equivalent amounts. Thus, if calcium replaces potassium, one calcium ion will replace two potassium ions.

2. Ions vary greatly in the ease with which they can be adsorbed or replaced by other ions. The ease or efficiency with which one ion replaces another ion is determined by its valence and its characteristic activity. Divalent ions are more potent than monovalent ions, with the exception of hydrogen ions. At equal ionic concentrations calcium and magnesium have greater replacing power than sodium and potassium. The activity of ions with the same valence also varies and seems to depend on the relative size of the hydrated ions. The smaller the hydrated ion, the greater its activity. When the following hydrated monovalent ions are placed in order of increasing size, we have Rb^+ ,

NH_4^+ , Na^+ , Li^+ . The decrease in replacing power of these ions follows the same order. If we compare the activity of these ions with the activity of some of the common divalent ions and with hydrogen ion, the descending order of replacing ability is found to be $\text{H}^+ > \text{Sr}^{++} > \text{Ba}^{++} > \text{Ca}^{++} > \text{Mg}^{++} > \text{Rb}^+ > \text{K}^+ > \text{NH}_4^+ > \text{Na}^+ > \text{Li}^+$. A comparison of the ease of adsorption of two of the ions in this series is shown in the work of Joseph and Oakley. They found that six times as many calcium ions as sodium ions were adsorbed when equimolar solutions of salts of these ions were allowed to percolate through certain soils.

3. Base-exchange reactions follow the law of mass action. An example with which the reader is familiar is found in the operation of the permutite water softener. Calcium and magnesium will replace sodium from the permutite as hard water is passed through the softener. But the reaction can be reversed and the permutite regenerated by forcing a saturated sodium chloride solution through it.

To soften water:



To regenerate the permutite:



Another illustration of the operation of the law of mass action can be found in base-exchange reactions. If a substance is present that forms an insoluble compound with an adsorbed positive ion, such a cation will be replaced. For example, in the presence of phosphate and oxalate ions, calcium will be replaced by sodium and potassium, for calcium phosphate and oxalate are insoluble but the sodium and potassium compounds are soluble.

Base-exchange capacity. If a soil sample is treated with ammonium acetate solution, a reaction will take place in which NH_4^+ ions displace the previously adsorbed cations. The quantity of ammonium ion remaining in the soil after adequate washing may be determined and expressed as milliequivalents per 100 grams of soil. (A milliequivalent of an ion will combine with or replace 1 milligram of hydrogen.) The values so ob-

tained are taken as a measure of the *exchange capacity* of a soil. Exchange capacity is another term for cation-adsorbing ability.

The exchange capacity of a soil varies with its composition. For example, the exchange capacity of clay is about 10 to 100 milliequivalents per 100 grams. The importance of humus in the base-exchange complex is indicated by its high exchange capacity, 400 milliequivalents per 100 grams.

Anion exchange. Since the exchange of cations on soil colloids is called base exchange, the exchange of anions has been called *acid exchange*. However, acid exchange is not so well understood as base exchange. It is certain that most anions are not held by soil colloids as tightly as cations, for they are readily lost from the soil by moderate leaching. Nevertheless it is believed that anion as well as cation exchange does take place between the soil solution and soil colloids.

THE SOIL SOLUTION

The components of soil, both organic and inorganic, appear to be difficultly soluble in water. However, soil compounds are soluble to a limited extent in the moisture which surrounds soil particles. The quantity of such dissolved material is always small at any one time, and the concentration of the solution varies greatly with the quantity of water present.

If soluble materials present in the soil solution are removed by plants or other agencies, the supply of plant nutrients is replenished by the dissolving of more material from the soil. Ions present in the soil solution are in equilibrium with ions adsorbed on the soil colloids. The maintenance of this ionic equilibrium by adsorption and release of nutrient ions is one of the important functions of soil colloids.

The soil solution of fertile soils contains a sufficient quantity of nutrients to supply the plants growing upon them. Excessive depletion because of overcropping or leaching leads to lower crop yields, and nutrients must be restored by the addition of manure or artificial fertilizer or by allowing the soil to lie fallow.

ABSORPTION OF PLANT NUTRIENTS

Our knowledge of the way in which nutrients are absorbed from the soil solution by plants is still fragmentary. Normal osmosis will account for the passage of water into root cells, for the soil solution is more dilute than the root cell solution. However, for the same reason the root cells should not be able to absorb molecules or ions from the more dilute soil solution. Nevertheless this anomalous movement of solutes into plants against a concentration gradient does take place. Such a phenomenon requires a source of energy.

The apparently abnormal behavior of soil particles in solution is known to have some connection with plant respiration. When plants die, the movement of solutes into plant cells ceases. Even living plants no longer absorb nutrients when placed in an atmosphere of nitrogen or carbon dioxide. Respiration must take place if plant root cells are to secure molecules and ions from the soil solution.

In our study of respiration and biological oxidation we have seen that the end products of this process are carbon dioxide, water, and energy. Carbon dioxide is given off by plant roots and is known to play an important part in plant-soil relationships. However, the principal effect of respiration on nutrient absorption may not be due to the action of carbon dioxide. It is more probable that the energy resulting from biological oxidation makes possible the anomalous diffusion of nutrients into root cells. Conceivably the energy produced may affect absorption by altering the charge on the semipermeable membranes of root cells.

The most important ions present in the soil solution are:

H^+	OH^-
NH_4^+	NO_3^-
K^+	H_2PO_4^-
Ca^{++}	HCO_3^-
Mg^{++}	HPO_4^{--}
Cu^{++}	CO_3^{--}
Zn^{++}	SO_4^{--}
Fe^{+++}	BO_3^{---}
Mn^{+++}	PO_4^{---}

Ions differ greatly in the rapidity with which they are absorbed by the growing plant. Of the common cations, potassium is most readily absorbed, and calcium the least readily absorbed. Of the common anions, the nitrate ion is absorbed most readily, and the sulfate ion appears to be the least readily absorbed.

The absorption of a given ion by a plant is influenced by the presence of other ions. For example, the intake of potassium is more rapid when supplied as the nitrate than when supplied as the sulfate.

One step in the process by which plants obtain cations from the soil is believed to be an exchange, principally between the hydrogen ions from the plant roots and the cations from the soil complex. The absorption of cations is thought to take place in two ways. In the first way the exchangeable ion is absorbed from the soil solution, but in the second the absorption is thought to take place through contact exchange between plant roots and soil particles. This direct contact exchange is believed to take place without having the ions become a part of the soil solution.

SOIL NUTRIENTS AND THEIR UTILIZATION BY PLANTS

The greatest amount of attention in the study of soil nutrients and their utilization by plants has been given to the four elements which are most frequently lacking in sufficient quantities for plant growth. These are calcium, nitrogen, phosphorus, and potassium.

Calcium. The calcium of the soil affects plant growth directly through its important role as an essential element and indirectly as this element determines in large measure the chemical, physical, and biological behavior of soils. Its importance from the standpoint of both soil fertility and plant growth has been recognized from the earliest times.

Absorption of nutrients by plants, leaching by water, and erosion result in the loss of calcium and other cations from soils. Such cations are replaced by hydrogen ions, and as a result the soil becomes more acidic. Calcium carbonate, hydroxide, or oxide (or a mixture of calcium and magnesium carbonate, hy-

dioxide, or oxide) is usually added to correct soil acidity. The added calcium compounds not only neutralize the acidity of soil but supply calcium required for the nutrition of plants.

The availability of the essential elements obtained by plants from the soil is affected by the hydrogen ion concentration of the soil. Increasing acidity below pH 6.5 is particularly important in limiting the availability of phosphorus, calcium, and magnesium, whereas at pH 's above 7.0 the availability of iron, copper, zinc, manganese, and boron decrease. Intelligent soil management, therefore, requires proper adjustment of the acidity of the soil, neither very acid nor very alkaline soils being desirable for most agricultural plants.

In order to achieve a soil reaction in the desired range, calcium compounds must be used judiciously. It is possible to overlime as well as to underlime a soil. Two other important effects from the addition of the proper amount of calcium to soils are evident. The fertility of well-limed soils is enhanced because of the favorable effect of calcium due to (1) increased activity of soil microorganisms and (2) improvement of the physical condition of soils.

The production of CO_2 by microorganisms of the soil and by plant roots plays a part in the solution and absorption of calcium. The carbonic acid resulting from the dissolving of CO_2 in soil water reacts with compounds of calcium to form soluble calcium bicarbonate.

Calcium and plant metabolism. The root systems of plants show the effects of calcium deficiency at an earlier stage than do the aerial parts. When a deficiency occurs, the size of the roots and the number of root hairs are greatly diminished, resulting in weak and stunted plants.

Calcium functions as a structural component of cell walls. Calcium pectate is one of the components of the middle lamella and, as such, plays an important role in the absorption and retention of ions by cells. In some manner, calcium appears to be necessary for the reduction of nitrates and the formation of proteins. Its absence results in the accumulation of both nitrates and carbohydrates. One of the functions attributed to calcium is the neutralization and precipitation of oxalic acid, a by-product of plant metabolism. This reaction protects the

plant against the toxic action of high concentrations of free oxalic acid.

The calcium content of plants varies with the plant species, the stage of growth, and the availability of calcium in the soil. Legume crops in general are much higher in calcium than grass crops. Because of the low calcium content of grasses, animals fed on hays grown on calcium-deficient soils often suffer from calcium deficiency. Deficiency diseases and malnutrition of cattle have been observed when the calcium content of plants used as feed was less than 0.25 per cent.

Nitrogen. Nitrogen is one of the elements essential to the maintenance of soil fertility. The lack of sufficient quantities of available nitrogen in soils has long been recognized as a limiting factor in crop production. An ample supply of readily available nitrogen stimulates vegetative growth of plants.

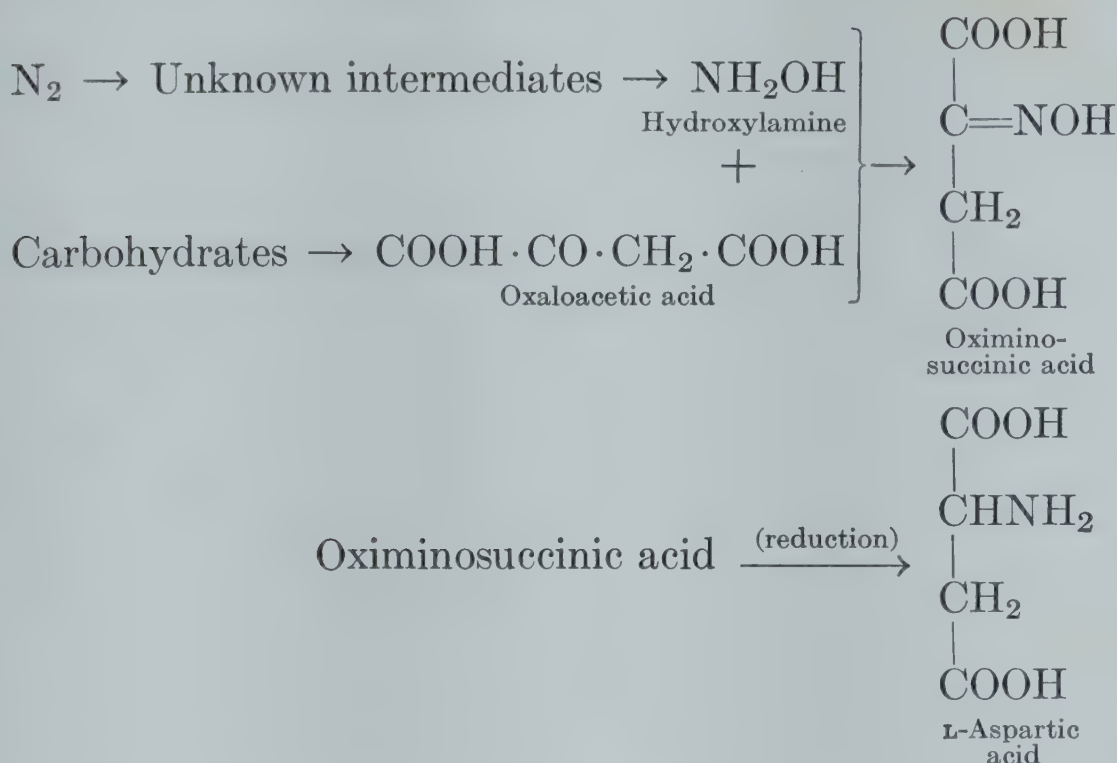
Most of the nitrogen found in soils occurs in combination with the soil organic matter. While in this form it is comparatively insoluble and not available for plant growth. However, the nitrogen of organic matter may eventually be liberated for utilization by plants through the action of microorganisms. All soils contain a small proportion of soil nitrogen in the form of relatively simple compounds such as amino acids, ammonium salts, and nitrates. It is from these compounds, particularly from ammonium salts and nitrates, that higher plants obtain their nitrogen. The total quantity of nitrogen found in mineral soils is usually quite low, varying from 0.1 to 0.5 per cent.

Atmospheric nitrogen is the ultimate source of soil nitrogen. The various processes by which this inert gas is changed into organic and inorganic compounds are biochemical. Some of these processes will now be considered.

Symbiotic nitrogen fixation. It has been known for a long period of time that legumes possess the power of enriching the soil with nitrogen, and that this nitrogen is obtained from the atmosphere through the agency of bacteria living upon them symbiotically. Symbiosis as applied to nitrogen fixation is derived from the Greek word for "living together." The bacteria, which belong to the genus *Rhizobium*, live in the root cells of the legume host, where their metabolic activity causes a swelling or nodule to form on the root. For this reason the organisms are commonly called root-nodule bacteria.

One of the facts established concerning the manner in which nitrogen is fixed is that the nodules excrete nitrogen chiefly as L-aspartic acid and its derivatives. It has also been established that hydroxylamine is one of the products formed, and that carbohydrate metabolism is closely related to nitrogen fixation. On the basis of these three facts a theory has been advanced which postulates that hydroxylamine unites with oxaloacetic acid to form an oxime which is then reduced to aspartic acid.

According to this theory the mechanism of biological nitrogen fixation would be as follows:



Since symbiotic nitrogen-fixing organisms are aerobic in character, a lack of oxygen inhibits nodule formation. The fact that nodules are formed in larger numbers near the surface of the soil is attributed to a greater oxygen supply at higher than at lower depths. Moisture conditions should be optimum for aeration and carbohydrate metabolism of the host plant. The optimal temperature for nitrogen-fixation by these organisms approximates 20° C, providing the pH of the environment is close to the neutral point. However, nodules are formed under any soil condition in which the legumes may grow.

Non-symbiotic nitrogen fixation. In addition to the symbiotic nitrogen-fixing organisms, there are two other important groups of nitrogen-fixing soil organisms. These function independently of higher plants. One of these has been given the generic term

of *Azotobacter*, the best known of which is *Azotobacter chroococcus*. These organisms are aerobic and will fail to develop under slightly acid conditions.

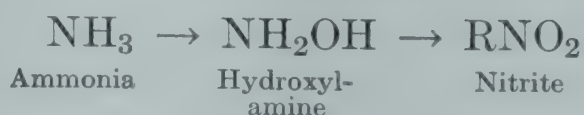
Another group of non-symbiotic nitrogen-fixing bacteria is known by the species name of *Clostridium pastorianum*. These are anaerobic organisms and appear to function effectively in acid as well as in neutral soils and under conditions that are unfavorable to most species of *Azotobacter*.

Ammonification. The production of ammonia from organic nitrogenous materials of soils is called *ammonification*. This process is an intermediate step in the process of nitrate formation by soil microorganisms. The organisms concerned with ammonification are the same fungi, bacteria, and actinomycetes that decompose organic matter in soils.

A neutral solution of albumin, if inoculated with ammonifying organisms, becomes alkaline in a very short time. This is due to the accumulation of ammonium carbonate in the solution. At the same time, proteoses, peptones, peptides, amino acids, and fatty acids are formed and the albumin content correspondingly decreases. This degradation of proteins is brought about by active proteinases secreted by the organisms themselves. In general, the degradation of proteins under soil conditions proceeds along the same lines as the degradation produced by enzymes and acids under laboratory conditions. But in the soil further changes take place after hydrolysis has occurred. Some of the compounds formed in addition to ammonia are carbon dioxide, methane, amines, organic acids, aldehydes, skatole, indole, and hydrogen sulfide.

The most important ammonifying organisms are aerobic and require a good supply of oxygen and moisture. The most favorable pH for their action seems to be pH 6.0 to pH 8.0.

Nitrification. The conversion of ammonia nitrogen to nitrate nitrogen in soils is accomplished in two steps by two classes of bacteria. The first step, which is carried out by *Nitrosomonas* and *Nitrosococcus* organisms, changes ammonia to nitrites. This takes place in the following way:



The second step is carried out by *Nitrobacter* and oxidizes nitrites to nitrates.



The final oxidation to nitrates takes place more rapidly than the formation of ammonia or nitrites, with the result that very little ammonia or nitrite nitrogen is present at any one time. The presence of ammonia nitrogen in amounts more than a few parts per million of soil is evidence that conditions are unfavorable for nitrification. This is commonly the case in acid soils rich in organic matter or when the moisture content of the soil is too high. Nitrification is an aerobic process, the work of autotrophic bacteria whose energy is obtained from the oxidation involved in this change. The minimal, optimal, and maximal temperatures for the process are about 5° C, 25 to 35° C, and 55° C, respectively. Although nitrification proceeds most rapidly in a neutral or a slightly acid medium, it is now known that it can occur, though more slowly, in soils that are definitely on the acid side of neutrality. The presence of moisture is, of course, essential for all microbial actions.

Nitrogen and plant nutrition. Nitrogen is an essential constituent of all amino acids, proteins, and enzymes. It is also an essential part of the molecules of chlorophyll, nucleic acids, and certain carbohydrates.

Nitrogen stimulates cell reproduction and thus favors the vegetative rather than the reproductive phase of plant growth. A plant well supplied with nitrogen produces a well-developed leaf system which, because of its increased surface, favors carbon dioxide assimilation.

An oversupply of nitrogen, not properly balanced with other nutrients, produces a watery, succulent growth. This type of growth may be desirable in leafy vegetables, but it is undesirable for grains. Excessive nitrogen also delays maturity and seed development. Nitrogen as well as calcium must be added judiciously to the soil to secure optimal results in plant growth.

Phosphorus. Phosphorus is found in every living cell and is essential in both plant and animal nutrition. Its importance in agriculture is indicated by the fact that low crop production is

due more often to a lack of phosphorus than to the lack of any other element.

The phosphorus content of soils, expressed as P_2O_5 , varies from 0.03 to 0.40 per cent. Phosphorus occurs in both the inorganic and the organic fractions of soil. The phosphorus of soil organic matter is released and made available to plants by the action of bacteria. Soil-forming rocks contain phosphorus in such minerals as chlorapatite, $Ca_5(PO_4)_3Cl$, and fluorapatite, $Ca_5(PO_4)_3F$. Such minerals are changed to more soluble compounds by chemical forces.

There is little water-soluble phosphorus present in soils at any one time, owing to the fact that soluble phosphorus compounds are rapidly changed to less soluble forms. Soluble iron and aluminum compounds, in particular, react with soluble phosphorus to form compounds of low solubility.

A slightly alkaline reaction favors the ionization of a phosphate into HPO_4^{--} ions, whereas a slightly acid reaction favors the formation of $H_2PO_4^-$ ions. The latter ions are more readily absorbed and utilized by growing plants. Phosphate ions are presumably adsorbed by the colloidal complex, and a reversible ionic exchange between phosphate and hydroxyl ions takes place.

Phosphorus aids the fertility of soils through its action as a nutrient for soil organisms. Some organisms require large quantities of phosphorus for normal growth; all organisms require certain amounts of the element.

Phosphorus and plant nutrition. Phosphorus is a constituent of many proteins such as nucleoproteins and phospholipids such as lecithin. Since phosphorus is necessary for the formation of nucleoproteins it is necessary for cell division. Phosphorus is also essential in carbohydrate metabolism, for as we have seen in an earlier chapter this metabolism proceeds by the formation of a series of intermediate phosphates such as hexose phosphates. It is also known that phosphorus plays an important role in the transfer of energy through the formation of energy-rich phosphate bonds.

Phosphorus favors seed formation and maturity of plants. In this respect it is antagonistic to nitrogen, which favors vegeta-

tive growth and delayed maturity. Because of its limited solubility in the soil solution, an excessive phosphorus content of plants is seldom a problem. However, low phosphorus not only affects plant growth and metabolism but gives rise to phosphorus deficiency in animals who subsist on such plants.

Potassium. When we examine the overall composition of soils we find that the total quantity of potassium is comparatively high. The upper soil layer averages about 2 per cent K_2O . This means that there are 40,000 pounds per acre of K_2O in the plowed layer, whereas calcium, nitrogen, and phosphorus average about 20,000, 4000, and 3000 pounds, respectively. It would seem that potassium should not be a limiting factor for plant growth in the average agricultural soil.

Nevertheless few agricultural soils under continuous intensive cultivation contain sufficient potassium to satisfy the needs of plants. Additions of soluble potassium salts must be made. The necessity of such additions is due to the fact that most potassium minerals of soils are difficultly soluble. These minerals release potassium so slowly that plants annually remove more of the element than is made available from soil compounds.

Experience has indicated that the availability of potassium to the growing plant depends in large measure on the extent of root development and on rainfall. When the rainfall is both ample and well distributed, the movement of potassium from the soil to the interior of the plant is facilitated. The intake of potassium, regardless of the fertilizer treatment, may be seriously interfered with by a drought or by unlimited rainfall during the growing season.

Potassium and plant nutrition. Potassium has a number of important functions in plant metabolism. It is present in soluble compounds, particularly as salts of organic acids, and it is highly mobile. Potassium is found in the cell sap and plays an important part in the water economy of plants through the effect of its soluble compounds on osmotic pressure.

Potassium aids the oxidative reactions of plants by acting as a carrier for iron, which in turn is required by a number of important oxidative enzymes. The assimilation of carbon and the formation of carbohydrates has been shown to be dependent on

the presence of potassium. One of its functions in carbohydrate metabolism is to form the K salts of the phosphorylated intermediates. High carbohydrate plants such as sugar beets contain a high potassium content. Protein metabolism in plants also requires the presence of an ample supply of potassium.

In the early stages of growth, plants supplied with limited amounts of potassium may show normal development. Later the relatively small amount of potassium migrates from older tissues to younger tissues. This is followed by a migration of nitrogen to the younger tissues. These migrations result in premature aging of older leaves when potassium is present in sub-optimal amounts.

OTHER MACRONUTRIENT ELEMENTS

In addition to calcium, nitrogen, phosphorus, and potassium, plants require three other mineral elements in larger than micro amounts. These elements are magnesium, sulfur, and iron. The seven elements mentioned above are called *macronutrient* elements.

Magnesium is a constituent of the chlorophyll molecule and hence is essential to all green plants. Soils deficient in magnesium are found particularly in the eastern states.

Sulfur deficiency in soils seldom appears, for the atmosphere contains a considerable quantity of sulfur which is brought to the soil by rain; and commercial fertilizers usually contain substantial quantities of this element. Sulfur is a constituent of proteins and therefore is essential for plant growth. Plants are able to utilize inorganic sulfates for protein formation, whereas animals are not able to do so.

Iron deficiency in plants results in a lack of chlorophyll and chlorosis. The part that iron plays in the production of chlorophyll is not understood, but it is known that iron is a part of several important oxidative enzymes. Soils are seldom actually deficient in this element, but it may be present in a form that is unavailable to plants. Such a condition may exist when the soil is alkaline due to overliming.



FIG. 10. The effect of various mineral deficiencies on the growth of tobacco: *A*, adequate nutrition; *B*, nitrogen deficiency; *C*, phosphorus deficiency; *D*, potassium deficiency; *E*, boron deficiency; *F*, calcium deficiency; *G*, magnesium deficiency. (Courtesy of J. E. McMurtrey, Jr., United States Department of Agriculture.)

MICRONUTRIENT ELEMENTS

The *micronutrient* elements—elements required for plant growth but only in very small amounts—are copper, manganese, zinc, boron, and molybdenum.

Copper is essential to plant growth for it is a constituent of several important oxidative enzymes. Copper deficiency in plants has been found to occur in certain areas of Florida, Oregon, Indiana, and other states.

D. E. Green of the University of Wisconsin has postulated that any element required for plants or animals in micro amounts will be found to function as a part of an enzyme system. *Manganese* is apparently such an element. It is known that manganese is required for the growth of plants and that it is a limiting factor in crop production in several areas of the United States. Manganese, like iron, is not often deficient in soils but becomes insoluble under alkaline conditions. Manganese is present in

such large amounts in soils of Hawaii and Puerto Rico that plants grown on these soils sometimes suffer from manganese toxicity.

Zinc is known to be a part of the molecule of the enzyme, carbonic anhydrase. Zinc deficiency has been noted in fruit trees, tomatoes, corn, and other plants.

Boron is required in very small amounts but is toxic at only slightly higher concentrations. With a number of plants this difference in concentration is only a few parts per million. Boron deficiency is first noted by abnormal development of terminal buds.

Molybdenum has been proved to be essential for the growth of plants, but its function is uncertain. It has been found that small quantities of sodium molybdate stimulate symbiotic nitrogen fixation.

A discussion of the relation of carbon, hydrogen, and oxygen to plant metabolism is included in the following chapter.

II • Fertilizers

A system of intensive agriculture which removes most of the plants from the farm on which they are produced sooner or later results in decreased crop yields unless certain practices in soil management are followed. In order to continue to produce good yields of crops, additional nutrients must be made available, either directly or indirectly.

Elements required for the maintenance of soil fertility are stored in soil minerals from which they may be released by growing plants. A system of rotation can be followed in which one crop is raised for the purpose of mining nutrient elements from soils. This plant crop when returned to the soil furnishes organic matter as well as available nutrients to help maintain soil fertility.

One of the best ways to make use of farm crops for the maintenance of soil fertility is to feed the plants to animals and to return the manure to the soil. The use of farm manure as a fertilizer and factors affecting its value will be discussed later in this chapter.

However beneficial the practice of returning plants to the soil may be, often it is not feasible to rely on this one method for the maintenance of soil fertility. Other sources of nutrient elements must be added. Materials that supply the lacking mineral elements are called *fertilizers*.

COMMERCIAL FERTILIZERS

The usual meaning of the term fertilizer in commerce includes only the principal nutrient elements, nitrogen, phosphorus, and potassium, but does not include calcium, magnesium, sulfur, or micronutrient elements. However, commercial fertilizers contain

many elements in addition to the principal three given above. Thus, sulfur, chlorine, sodium, calcium, magnesium, and small quantities of many other elements may be present. Nevertheless, fertilizers are sold on the basis of their content of nitrogen, phosphorus, and potassium. This content is stated as per cent N, P_2O_5 , and K_2O , given in that order. Therefore, a fertilizer the composition of which is given as 6-9-12 contains 6 per cent nitrogen, 9 per cent phosphorus, calculated as P_2O_5 , and 12 per cent potassium, calculated as K_2O .

Owing to the above conventions in showing the analysis, it is customary to speak of fertilizer materials and mixtures as containing nitrogen, phosphoric acid, and potash. Until recent years it was also customary to speak of the ammonia instead of the nitrogen content of fertilizers. These terms have no significance with respect to the compounds in which the elements are found in fertilizers. Each of the three elements may be present in several different compounds.

Nitrogenous fertilizers

Four types of nitrogenous fertilizers are available. These are nitrates, ammonium compounds, organic compounds of nitrogen, and animal and vegetable residues.

Nitrates. The most common nitrate available for fertilizer is *sodium nitrate* (nitrate of soda). It occurs in a mixture of various salts, called caliche, found in large deposits in northern Chile. After purification by selective crystallization, the nitrate of soda is melted and sprayed into an enclosed chamber. The drops, on cooling, solidify into small shotlike pellets, a form that is convenient for packaging and handling. This pellet form contains about 99 per cent sodium nitrate.

Potassium nitrate is a desirable source of both nitrogen and potassium. However, the supply of nitrate of potash is quite limited. The principal source is caliche, which contains 2 to 3 per cent potassium nitrate. The commercial product derived from caliche is a crude grade which contains both sodium and potassium nitrate and has a nitrogen content of about 14 per cent and a potash content (K_2O) of about 15 per cent. Small amounts of purer KNO_3 are also available for fertilizer use.

Calcium nitrate, also known as nitrate of lime and Norwegian saltpeter, is an excellent source of quickly available nitrogen, containing about 16 per cent nitrogen and 34 per cent calcium, expressed as CaO. It is much more hygroscopic than either of the above nitrates, and because of this it is more difficult to apply to the soil. Owing to this fault it is not entirely satisfactory as a fertilizer, and its use has been limited. It is made in Norway and Germany by the neutralization of nitric acid with calcium carbonate.

Ammonium nitrate is also used as a nitrogenous fertilizer to some extent. Ammonium nitrate is a hazardous material for it will ignite and detonate under certain conditions.

Nitrates are easily soluble in water, and this characteristic ensures a rapid availability of nitrogen for plant growth. Excessive quantities of nitrates are undesirable from the standpoint of permanent soil fertility because they tend to inhibit the processes of nitrification and nitrogen fixation. The residual effect of sodium, potassium, and calcium nitrates is to decrease soil acidity, whereas ammonium nitrate increases soil acidity.

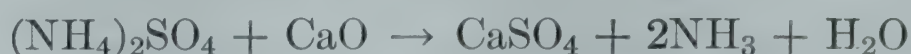
Ammonium compounds. Of all the nitrogenous fertilizer materials, *ammonium sulfate* is the most widely produced and used. Much of it is prepared by the neutralization of dilute sulfuric acid with ammonia, a by-product of destructive distillation of coal. In the *gypsum process* another common method for the preparation of ammonium sulfate, ammonia, and carbon dioxide are passed into a suspension of calcium sulfate, forming ammonium sulfate and calcium carbonate.



Ammonium sulfate tends to increase soil acidity, and soils fertilized with this source of nitrogen must be limed more frequently than those treated with sodium nitrate. Aqueous solutions of ammonium sulfate are acid, since the compound is a salt of the weak base NH_4OH and the strong acid sulfuric. An additional cause of increased soil acidity after applications of ammonium salts is a result of nitrification. The reactions can be shown as follows:



A popular method of explaining the acidifying effect of ammonium sulfate is to state that nitric and sulfuric acids are formed. Ammonium sulfate is a good source of nitrogen for most crops but not so satisfactory as nitrates for acid-sensitive plants such as beets, barley, and wheat. Other ammonium compounds sometimes used as nitrogenous fertilizers include *ammonium chloride*, *ammonium nitrate*, and several kinds of *ammonium phosphates*. Basic compounds such as lime or basic slag should not be mixed with ammonium salts, since a reaction takes place which liberates nitrogen as ammonia.

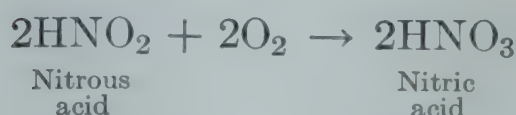
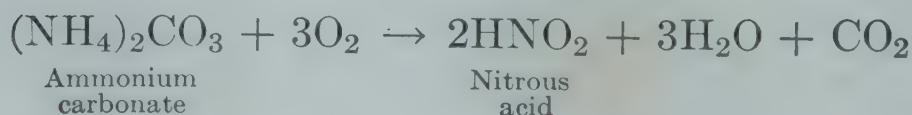
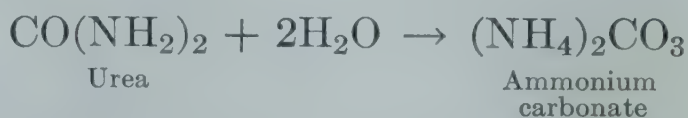


Anhydrous ammonia and various solutions containing a high ammonium content are used as a source of nitrogen by the manufacturers of mixed fertilizers. These sources of ammonia have been derived either from the air by direct synthesis or from the by-products of other reactions. They represent the cheapest available source of nitrogen. Anhydrous ammonia or *ammonia solutions* are utilized by mixing them with superphosphate. A series of reactions takes place which results in the formation of monoammonium phosphate, ammonium sulfate, and several other compounds.

Organic nitrogen compounds. *Urea* is manufactured by combining ammonia with carbon dioxide under pressure. The reaction takes place as follows:



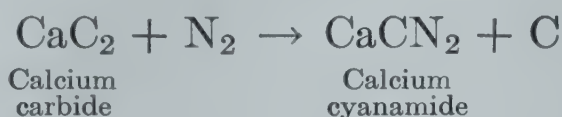
Urea is readily soluble in water and is probably absorbed by plants in small amounts. But much of the urea is converted to ammonium compounds and nitrates in the soil and is absorbed as ions of these compounds.



Urea is considered to be one of the best nitrogenous fertilizers because it is cheap, readily soluble, rapidly converted to ammonium and nitrate compounds, resists leaching, and increases soil acidity only slightly.

Calcium cyanamide, as produced for the fertilizer trade, is an impure, grayish black powder, containing a considerable amount of lime and free carbon. Pure calcium cyanamide (CaCN_2) is white and contains 35 per cent nitrogen. The commercial product contains about 21 per cent free CaO , 11 per cent free carbon, and smaller amounts of several other compounds.

The raw materials for the manufacture of calcium cyanamide are air, coal, and limestone. Nitrogen (prepared from air) and calcium carbide (produced from coke and lime) are heated at 1000°C to form calcium cyanamide.



Crude calcium cyanamide is particularly suitable for application to acid soil, owing to its high content of calcium oxide. When added to a soil having a pH of 7.0 or less, cyanamide is hydrolyzed to urea within a few days. It is this reaction which makes possible the use of cyanamide as a fertilizer, for the compound as such is toxic to plants. Plant toxicity is avoided if cyanamide is uniformly distributed and well mixed with the upper layer of soil and is applied at rates of 200 pounds per acre or less at least 10 days before planting.

Urëa and calcium cyanamide are called “non-proteid organic fertilizers” by fertilizer manufacturers to distinguish these products from fertilizers of animal and vegetable origin.

Animal and vegetable residues. Nitrates, ammonium compounds, and non-protein organic fertilizers are readily soluble in water and rapidly removed from the soil. It is often desirable to add nitrogen in a form which is less soluble and is more slowly available to plants. The nitrogen of animal and vegetable residues, found principally in proteins, is such a source.

Fish scraps, tankage, and dried blood are animal residues formerly used in large amounts as fertilizers. These materials are now used in animal feeds and can command a better price for this purpose; hence their use as fertilizers has declined. Fish scrap contains from 5 to 10 per cent nitrogen and from 4 to

7 per cent P_2O_5 . Dried blood contains from 9 to 14 per cent nitrogen.

Cottonseed meal is one of the most widely used plant residues. It is a by-product resulting from the extraction of oil from cottonseed. Analysis shows that it contains about 7 per cent nitrogen, 2.5 per cent P_2O_5 , and 2 per cent K_2O . Because the better grades of cottonseed meal are used for stock feeding, the price per unit nitrogen is higher than the inorganic nitrogen materials.

The residues remaining after oil extraction from several other seeds are also used as fertilizers. These include *castor pomace* from the *castor bean*, *linseed meal*, and *soybean oil meal*.

A product becoming more available as a fertilizer is *sewage sludge*. This is a product resulting from the treatment of city sewage. It is called *activated sewage sludge* if in the process of manufacture it has been inoculated with microorganisms and aerated. Dried activated sewage sludge is produced and marketed by the sewage commission of the city of Milwaukee under the trade name *Milorganite*. Other cities are also producing similar products. Activated sewage sludge contains about 4 to 6 per cent nitrogen and from 2.5 to 4.0 per cent available phosphoric acid.

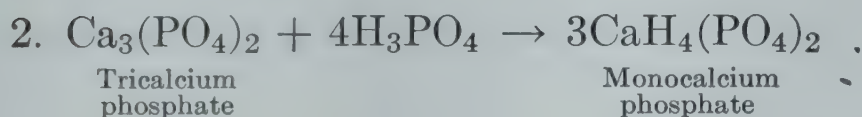
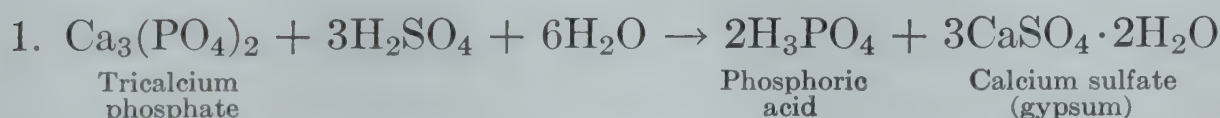
Phosphate fertilizers

The importance of phosphates is indicated by the fact, stated in a previous chapter, that low crop production is due more often to a lack of phosphorus than to the lack of any other element. All phosphatic fertilizers contain phosphorus as salts of *o*-phosphoric acid.

Rock phosphate. Rock phosphate, consisting largely of $Ca_3(PO_4)_2$, occurs in large deposits throughout the world. In the United States deposits of commercial importance occur in Florida, Tennessee, Idaho, Montana, Utah, and Wyoming. A limited amount of finely ground rock phosphate is used for direct application as a fertilizer. However, it is not a readily available source of phosphorus because the solubility of tricalcium phosphate is very low. The use of rock phosphate is not recommended when monocalcium phosphate, ammonium phosphate,

or other more soluble forms can be obtained. The chief use of rock phosphate is in the preparation of other forms of phosphatic fertilizers.

Monocalcium phosphate. Monocalcium phosphate is one of the products formed when rock phosphate is treated with sulfuric acid.



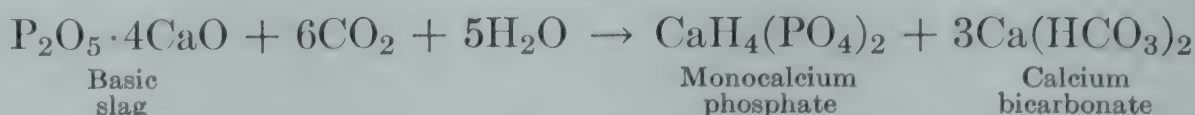
A small amount of dicalcium phosphate, $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$, is also formed during the reaction. The mixture of products formed from the above reactions contain about 50 per cent gypsum and 26 per cent monocalcium phosphate. This mixture is sold as the fertilizer called *superphosphate* or *acid phosphate* and contains 16 to 20 per cent available P_2O_5 . An application of monocalcium phosphate may result in a temporary increase of soil acidity.

Concentrated superphosphate, called double or treble superphosphate, containing from 40 to 48 per cent available P_2O_5 , is also manufactured. This product results when the calcium sulfate is removed in reaction 1 above and free phosphoric acid reacts with rock phosphate as in reaction 2. The mixture contains very little gypsum but may have a considerable quantity of free phosphoric acid.

We have already mentioned the fact that *monoammonium phosphate* is produced when superphosphate is treated with anhydrous ammonia or ammonia solutions. The mixture of products obtained by this reaction is marketed as *ammoniated superphosphate* and contains from 16 to 18 per cent P_2O_5 .

Basic slag, a by-product of the steel industry, is a relatively insoluble form of phosphatic fertilizer. But its phosphorus is far more available than that of rock phosphate. It contains from 8 to 25 per cent P_2O_5 , as much as 50 per cent CaO , and varying amounts of Fe_2O_3 , SiO_2 , and other compounds. In terms of its principal constituents its composition is sometimes given as $\text{P}_2\text{O}_5 \cdot 4\text{CaO}$.

In the soil, basic slag is made available by the following reaction:



Bones are used as the parent material for several different products sold as phosphatic fertilizers. *Raw bone meal*, *steamed bone meal*, and *precipitated bone* are such products. The form of phosphorus present in bones is thought to be $\text{Ca}_3(\text{PO}_4)_2$. Dicalcium phosphate results when precipitated bone is made as a by-product of glue manufacture. Both raw and steamed bone meal contain nitrogen as well as phosphorus, but the cost of these elements per unit is higher when purchased in bone meal than when bought in other forms.

The term *available phosphorus*, or available phosphoric acid, as used in the fertilizer industry has a definite meaning. Available phosphorus is the sum of the water-soluble phosphorus and the ammonium citrate-soluble phosphorus. The value for available P_2O_5 obtained in this manner approximates the amount of phosphorus in a fertilizer which will be usable by plants.

Potash fertilizers

Deposits of potassium salts are found in various parts of the world, but only those found in France, Germany, and the United States are mined extensively. Sources of potash in the United States include the natural brines of inland lakes in Nebraska and California, and deposits of sylvite (KCl) and kainite ($\text{MgSO}_4 \cdot \text{KCl} \cdot 3\text{H}_2\text{O}$) in New Mexico. Greensand found in eastern states, particularly New Jersey, is present in quantities sufficient to supply the potassium needs of the United States for hundreds of years. Many of these deposits are near or on the surface and can be mined by power shovels. Greensand is classified as glauconite ($\text{KFeSi}_2\text{O}_6 \cdot n\text{H}_2\text{O}$), the potassium of which is very slowly available. Its greatest value seems to be as a raw material for the production of K_2SO_4 .

Potassium chloride (KCl) is the chief constituent of *muriate of potash*, *manure salts*, and *kainite*. The chloride is the most common form of potassium fertilizer and can be used on most

crops. However, the quality of some plants is adversely affected by the application of large quantities of chlorides. The sugar content of beets is lowered as is the quality of potatoes and the burning quality of cigar leaf tobacco when KCl is used as a fertilizer.

Sulfate of potash and *sulfate of potash-magnesia* are marketed as potassium fertilizers. The sulfates are somewhat more expensive than the chlorides, and their use is limited to special-purpose fertilizers in which the chloride is undesirable. The use of sulfate of potash-magnesia is particularly desirable when magnesium is lacking in the soil. This double salt of potassium and magnesium contains about equal amounts of potassium sulfate and magnesium sulfate. Its K_2O content (about 25 per cent) is about one-half that of sulfate of potash.

Other potassium fertilizers include *potassium nitrate*, *wood ashes*, *potassium phosphate*, and *tobacco stems*. *Sewage sludge* and the various seed meals such as *cottonseed meal* also contain small and variable amounts of potassium.

Potassium salts used as fertilizers are water soluble and therefore should be readily available to plants. However, potassium salts added to certain soils with high clay contents appear to be changed to forms in which the potassium is as unavailable as that in soil minerals. This fixation of added potassium is a problem that has occupied the attention of investigators for many years. Experimental workers have secured good results from potassium fertilization by making one extremely large application of potassium salts to satisfy the fixing power of the soil. In subsequent years this is followed by small additions of potassium fertilizers for each crop.

FARM MANURE

Farm manure is the solid and liquid excrement of animals, alone or mixed with litter. *Litter* is the straw, sawdust, or other absorbent material used in stables and barns. The term *manure* is sometimes applied to any fertilizer but is more often used, as it will be in this chapter, to mean farm manure. The value of manure for maintaining and improving soil productivity has been recognized from the earliest times. Its beneficial influence

on soil fertility is much greater than can be accounted for by its content of the three principal fertilizer elements, nitrogen, phosphorus, and potassium.

Composition of farm manure

Fresh farm manure consists of solid and liquid components, the former approximating 75 per cent and the latter 25 per cent of the total weight. As a rule about one-half the nitrogen and potassium and nearly all the phosphorus are found in the solid portion. The feces may contain a considerable amount of undigested matter in the form of the original compounds of the ration. However, the urine does not contain undigested feed but contains waste products from the organs and tissues of the body. The compounds eaten by animals are hydrolyzed in the alimentary tract through the action of digestive or bacterial enzymes. As a result much of the fresh excrement consists of compounds less complex than the compounds ingested. The manure as voided contains numerous microbial species, including fungi, actinomycetes, and, particularly, bacteria. A relatively large part of the total weight of manure consists of the bodies of living and dead bacteria. The most resistant constituents of feedstuffs from the standpoint of digestibility are the various plant lignins, although considerable quantities of cellulose and hemicellulose also escape digestion. In the intestinal tracts of animals lignin combines with proteins (chiefly bacterial proteins) to form lignoprotein complexes, called humus. The humus of manure apparently is identical to the humus found in soils. It has been estimated that 25 per cent of the organic matter of cow manure is humus.

The composition of fresh or decomposed manure varies greatly and can only be approximately stated. Its content of N, P, and K is comparable to that of a low-analysis fertilizer and may not exceed 0.5 per cent N, 0.25 per cent P_2O_5 , and 0.5 per cent K_2O . Only about one-half the nitrogen and potassium and about one-sixth the phosphorus are readily available to plants. Since the phosphorus is present in smallest amount and is less available than the other elements, it is evident that manure is not a

balanced fertilizer and should be supplemented with a source of phosphorus. The amount of phosphate fertilizers that should be added to a ton of manure depends upon the acre-rate of application of the latter. If 10 tons are to be spread on an acre of land, each ton should be reenforced with at least 50 pounds of 20 per cent superphosphate.

In addition to nitrogen, phosphorus, and potassium, manure contains substantial amounts of calcium, magnesium, and sulfur, and varying amounts of the micronutrient elements.

The factors that influence the composition of farm manure include kind of animal, age and ration of the animal, kind and quantity of litter, and storage conditions.

Kind of animal. The nitrogen, phosphorus, and potassium contents of manure vary with the species of animal from which it is derived, as shown in the following table:

THE COMPOSITION OF MANURE FROM DIFFERENT FARM ANIMALS
AS BASED ON ONE-TON QUANTITIES

(Data of Duley, *Missouri Station Bulletin* 166)

Animal	Weight of Manure, lb	Nitrogen, lb	Phosphorus, lb	Potassium, lb
Horse				
Solid	1632.2	8.06	2.12	3.26
Liquid	367.8	4.41	Trace	4.56
Total	2000.0	12.47	2.12	7.82
Cow				
Solid	1456.5	4.71	1.31	1.80
Liquid	543.5	5.16	0.06	4.29
Total	2000.0	9.87	1.37	6.09
Sheep				
Solid	1200.0	7.80	2.40	2.28
Liquid	800.0	13.44	0.10	14.08
Total	2000.0	21.24	2.50	16.36
Hog				
Solid	1290.3	7.74	2.58	4.77
Liquid	709.7	2.12	0.39	5.89
Total	2000.0	9.86	2.97	10.66
Hen	2000.0	23.00	8.10	7.46

The composition of manure is affected not only by species but by the utility of the animal. For example, on maintenance rations steers may excrete in feces and urine a quantity of mineral nutrients equal to that contained in the feed ingested, whereas milch cows may utilize some of these elements for the production of milk. It has been estimated that, for each 1000 pounds of milk, approximately 6 pounds of nitrogen, 2 pounds of phosphoric acid, and 2 pounds of potash are required.

Age of the animal. Growing animals excrete less of the fertilizing constituents of their feed than do mature animals. A young growing animal requires considerable quantities of calcium and phosphorus as bone-building materials, and measurable quantities of these and other constituents are retained for the synthesis of body tissues.

Ration of the animal. The composition and quantity of a given animal manure vary with the kind, quantity, and digestibility of the feed consumed. For example, the digestibility of corn may exceed 90 per cent with the result that 10 per cent or less of the total quantity consumed may be recovered in the manure. If overripe timothy hay, which has a low degree of digestibility, is fed to animals, about two-thirds of the total dry matter ingested may be excreted.

Kind and quantity of litter. The term *litter* is applied to material serving the double purpose of keeping animals clean and absorbing the water-soluble fertilizer constituents. Many different materials have been used for this purpose. These include cereal straws, cornstalks, hays, wood shavings, sawdust, peat, and other materials which may have relatively high absorbent qualities. Wood products such as sawdust and shavings contain more lignins and resins than do straw, hay, and cornstalks. These compounds are resistant to the action of microorganisms, and, as a result, wood products are decomposed slowly. Straw, hay, and cornstalks contain a greater amount of readily decomposed carbohydrates, more protein, and more minerals than do wood products.

A substantial part of the components of a ration are changed by the animal into products which differ from the original compounds. Hence the composition of manure, as voided, differs from the composition of litter, although ration and litter may

have a similar chemical analysis. Thus the ratio of litter to animal excreta will have an important effect on the composition of manure.

Storage of manure. Conditions under which manure is stored have a profound influence on its composition. Loss of manurial constituents may take place through two principal agencies: (1) leaching and (2) decomposition or fermentation.

Since a large proportion of the total nitrogen and potassium of farm manure is present in the form of soluble compounds, the possibility of their loss through leaching is usually great. An adequate amount of litter and tight floors prevent substantial losses from leaching in barns. But exposure of poorly built heaps to rainfall favors losses of soluble material. These losses are materially reduced if the heaps are built with steeply sloped sides and placed on concrete floors. Storage of manure in a roofed concrete pit will further reduce losses from leaching.

Not only does manure contain relatively large quantities of soluble compounds, but other materials are present which can be changed to soluble or volatile compounds as a result of decomposition or fermentation. Decomposition of stored manure is a continuous process, the rate of which varies according to environmental conditions.

Decomposition of manure

The liquid portion of manure is relatively rich in nitrogen, chiefly in the form of urea ($\text{CO}(\text{NH}_2)_2$). Through the activities of microorganisms, urea is broken down to ammonia and carbonic acid, which may unite to form ammonium carbonate ($(\text{NH}_4)_2\text{CO}_3$) or the acid carbonate (NH_4HCO_3). Both compounds are unstable, their instability being increased by increased temperatures, alkalinity, and drying. With the evolution of ammonia in the presence of a limited amount of carbon dioxide, the reaction of the decomposing material may reach pH 9.5. A slightly acid reaction, equal to pH 6.6, practically inhibits decomposition of the urea.

In addition to the readily decomposable urea, manure contains proteins. Some of these come from the feed or are present in the litter; others are of bacterial origin. Proteins undergo

hydrolysis with the liberation of amino acids, which, through biological oxidation, yield nitrogen in the form of ammonia or elemental nitrogen. Numerous other compounds arise as a result of protein degradation. These include indole, skatole, mercaptans, hydrogen sulfide, and amines, in addition to various organic acids.

The manurial solids contain considerable quantities of lignin, cellulose, hemicellulose, and related compounds. The rate and course of decomposition of the carbohydrates and related compounds varies with the environmental conditions. It is extremely rapid at relatively high temperatures with sufficient air supply and adequate moisture. An excess of moisture, through its interference with aeration, limits the reaction.

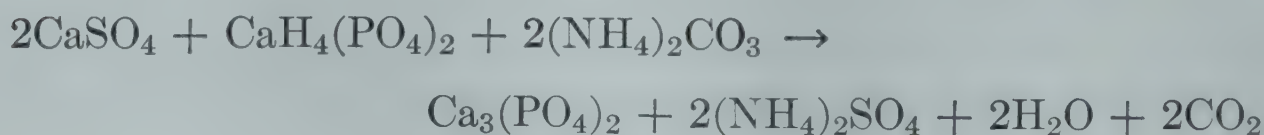
When anaerobic rather than aerobic conditions prevail, there is an accumulation of intermediate compounds resulting from the decomposition of carbohydrates, proteins, and related compounds which include various organic acids, ammonia, hydrogen, methane, and carbon dioxide. The acids tend to combine with ammonia, forming ammonium salts, and thus reduce the loss of nitrogen through the volatilization of ammonia. At the same time the *pH* of the manure is lowered.

During the decomposition of manure there is a simplification of numerous compounds and the synthesis of others. The final result of the synthesis of organic compounds is the formation of humus, a complex so resistant to decay that it may be considered almost stable.

Chemical treatment of manure. Concern over the rapid loss of ammonia from manure has led to the use of chemical preservatives. For a long period of time gypsum has been used for this purpose. The reaction results in the production of calcium carbonate and ammonium sulfate.



Superphosphate, which contains both gypsum and monocalcium phosphate, functions better than either of these compounds used alone, the reaction taking place according to the equation:



Tricalcium phosphate, unlike calcium carbonate, does not react with $(\text{NH}_4)_2\text{SO}_4$, and the above reaction is not reversible. Thus the addition of superphosphate accomplishes two results: it serves as a preservative for ammonia, and it supplies phosphorus to create a better balanced fertilizer.

Many materials having bactericidal properties have been employed as preservatives. These include formalin, chloropicrin, and the sulfates of iron, zinc, and copper. Strong acids, such as sulfuric, phosphoric, and hydrochloric, have been used to a certain extent. The latter compounds prevent the loss of ammonia through the formation of ammonium salts and change the *pH* to a value which inhibits fermentation. Whether the retention of ammonia compensates for the inhibition of bacterial life is open to question.

Effects of manure on soil

The beneficial effect of an application of manure as reflected by plant response may be very great. This effect may be apparent for only 1 year when manure is applied to gravelly or sandy soils but when it is applied to heavier soils profitable crop increases may be noted from 2 to 4 years later. At the Rothamsted Station the effects of eight yearly applications of 14 tons each were apparent 40 years after the last treatment.

Chemical effects. Additions of manure to a soil increase the quantity of mineral nutrients available for plant growth. The increase of available nutrients results in part from the liberation of plant nutrients carried by the manure and in part from materials made available from insoluble soil minerals. This is true especially for calcium, magnesium, potassium, manganese, and to a certain extent for other elements. The exchange capacity of manured soils is considerably increased over that of non-manured soils, reflecting the effect of added humus.

Physical effects. When manure undergoes a rapid decomposition in heavy soils, it improves the structure, allowing better aeration. The improvement in structure favors root development and plant growth and decreases soil erosion. Applications of well-decomposed manure improve light sandy soils by increasing their moisture-holding capacity.

The increased humus content following manurial treatment darkens the soil and increases heat absorption from the sun. The increased temperature of the soil is advantageous where early development or growth is desired.

Biological effects. The biological effects of a manure treatment may be more important than the physical and chemical effects. An application of manure supplies numerous bacteria and other organisms, a number of which will continue to thrive and to multiply. Such an addition stimulates the multiplication of many native soil organisms as well. The metabolism of soil organisms produces cementing substances called "bacterial cement" which causes aggregation of soil particles.

The fact that small applications of manure exert a much more favorable influence on plant growth than can result from its nutrient content alone has been attributed to the inoculation of the soil with desirable organisms and to the increased development of similar organisms peculiar to the soil. This increased bacterial activity is thought to result in the production of plant stimulants analogous to vitamins and hormones, designated as *phytamines* and *auximones*, respectively.

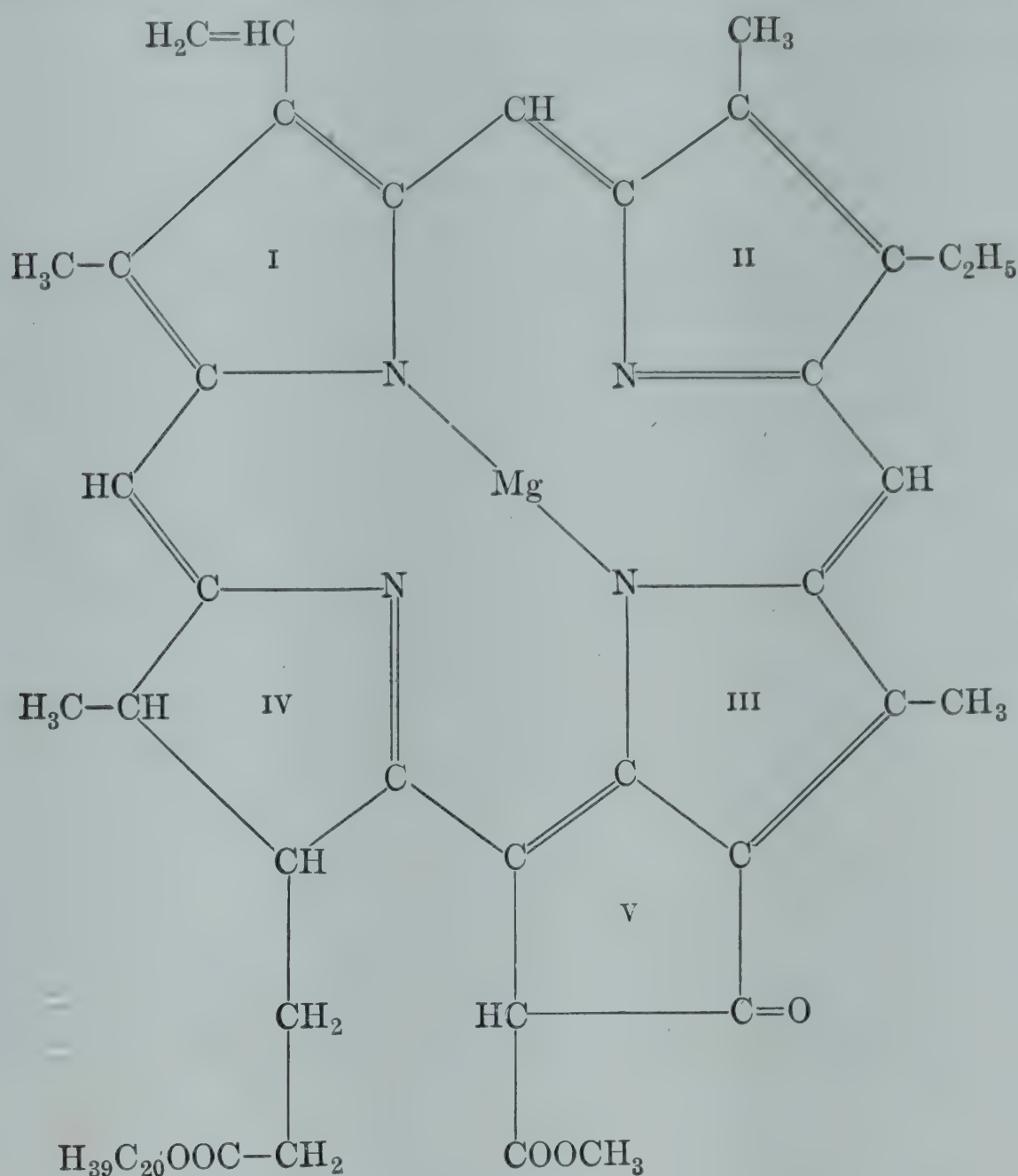
12 · Plant Metabolism

All living matter possesses the ability to carry on the processes of anabolism and catabolism. The processes of catabolism or breakdown of compounds in protoplasm are quite similar in all living organisms. Studies of the biochemistry of higher plants, animals, and microorganisms have shown that such reactions as hydrolysis of carbohydrates, lipids, and proteins are common to all. However, there is a decided difference among organisms as to their ability to synthesize the compounds of protoplasm, although the final products may be similar. Some organisms require only carbon dioxide, water, and other inorganic substances in order to produce the myriad compounds found in protoplasm. Other organisms need preformed organic compounds of various kinds in order to build their protoplasmic constituents. The first group of independent, self-nourishing organisms are called *autotrophs* and include higher green plants, algae, and chemosynthetic bacteria. The dependent group, called *heterotrophs*, include animals, man, fungi, and most bacteria.

CARBOHYDRATE METABOLISM

After the seed of a green plant has germinated and the seedling has become established in the soil or other media, the young plant becomes autotrophic. Organic compounds are no longer needed for its growth, and it requires only light, carbon dioxide, water, oxygen, and the ions of the elements discussed in the chapter on soils. The energy required for the building of energy-rich organic compounds from energy-poor material is furnished by light. The process is called photosynthesis.

Photosynthesis. Various investigators have shown that the primary products of photosynthesis are carbohydrates. The conversion of light energy to chemical energy contained in carbo-



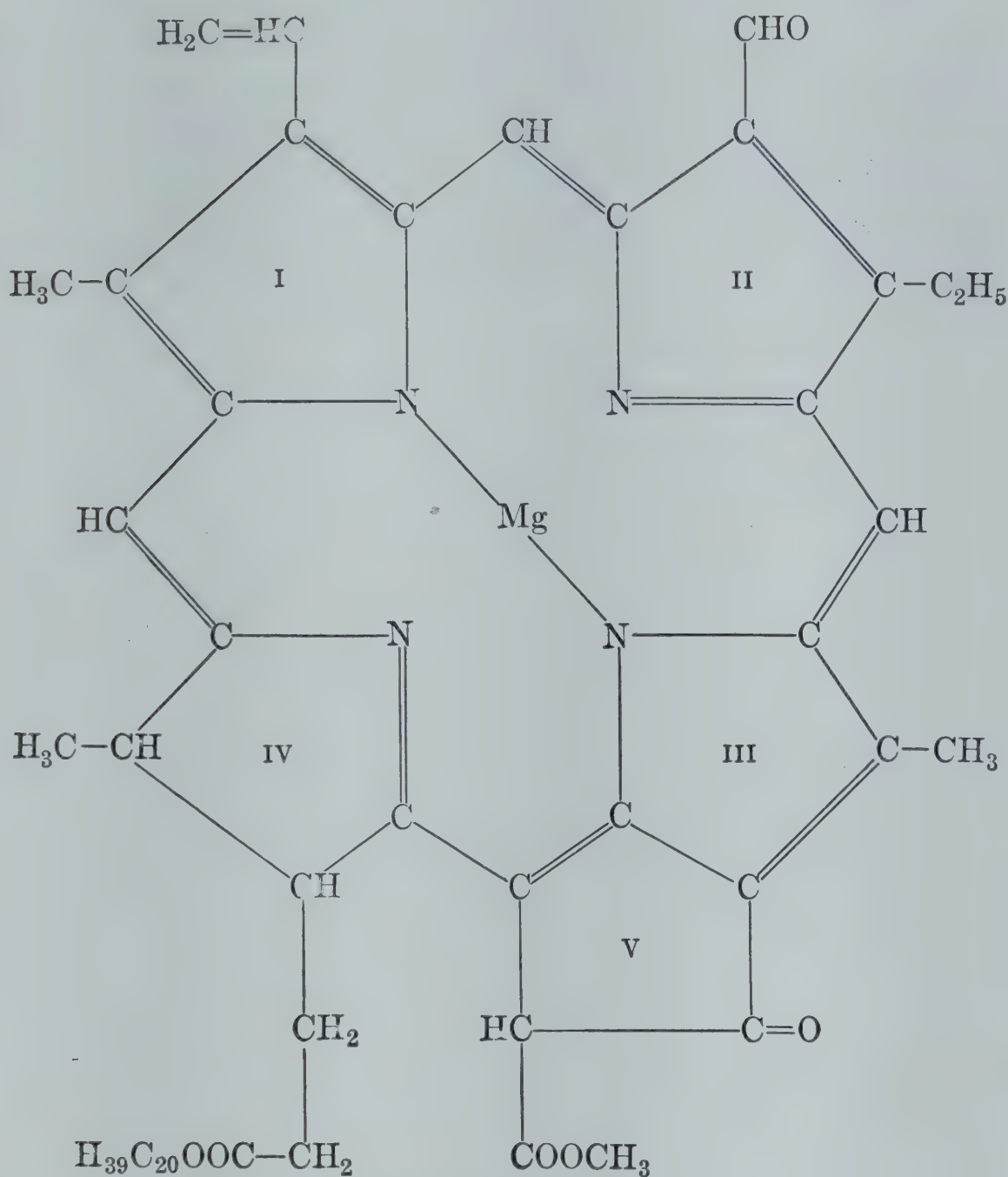
Chlorophyll *a*

hydrates and derived products is a characteristic of plants containing the green chlorophyll pigments. Two of these pigments are present in higher plants: chlorophyll *a* ($\text{C}_{55}\text{H}_{72}\text{O}_5\text{N}_4\text{Mg}$), and chlorophyll *b* ($\text{C}_{55}\text{H}_{70}\text{O}_6\text{N}_4\text{Mg}$).

The formulas of the two pigments are similar; the methyl group attached to ring II in chlorophyll *a* is replaced by an aldehyde group in chlorophyll *b*. In general there are about

three molecules of chlorophyll *a* to one molecule of chlorophyll *b* in higher plants.

The chlorophyll pigments are esters of a complex dicarboxylic



Chlorophyll *b*

acid. The alcohols, which are released on hydrolysis, are phytol ($\text{C}_{20}\text{H}_{39}\text{OH}$) and methanol. Phytol can be reversibly replaced under the catalytic influence of the enzyme chlorophyllase. Phytol has been found to be of biological importance in both plants and animals, since it is now known to be a part of vitamin K.

The net overall reaction of photosynthesis can be shown by the equation:



The individual reactions which make up the overall equation shown above are only partially known. Some of the known reactions will be considered.

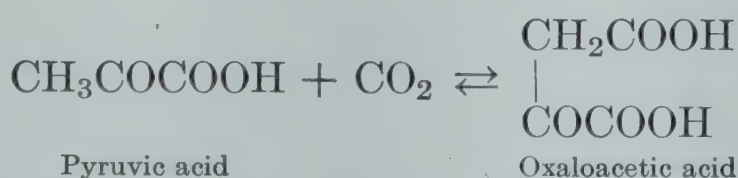
Studies with heavy oxygen have shown that the oxygen given off in photosynthesis comes from water. This means that for the production of six molecules of O_2 , twelve molecules of H_2O



are required. It is probable that chlorophyll is required in this reaction, but its mode of action is unknown. The production of oxygen from water takes place in light and ceases shortly after green plants are placed in the dark.

The function of hydrogen produced from water is to reduce carbon dioxide to carbohydrate, six molecules of water being formed. The reduction of CO_2 , following its fixation, has been shown to be an enzymatic reaction which will proceed in the dark. Thus the photosynthetic process is divided into a light reaction phase requiring chlorophyll and a dark reaction phase catalyzed by enzymes. Both phases probably consist of many steps. It has been postulated that the reduction of CO_2 by a series of reactions takes place in a manner similar to the reversal of carbohydrate oxidation.

Assimilation of carbon dioxide takes place by the reaction of CO_2 with keto acids such as pyruvic. These reactions are



catalyzed by carboxylase enzymes and are a reversal of the reactions by which carbon dioxide is liberated in biological oxidations.

A reversal of the carbohydrate metabolism reactions from the direction of the formation of CO_2 and H_2O to the formation of hexose phosphates suggests that fructose, glucose, or starch should be the first carbohydrate formed. However, Calvin and

Benson have shown by radioactive tracer studies that sucrose may be the first carbohydrate produced.

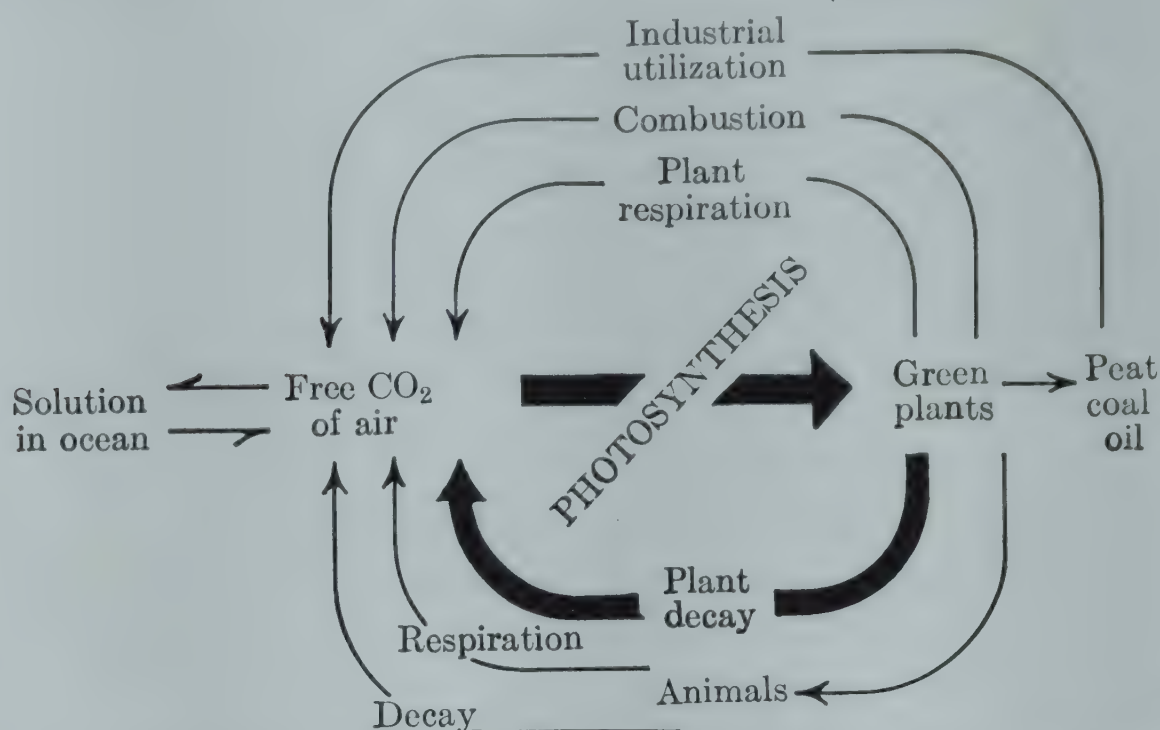


FIG. 11. The carbon cycle. Photosynthetic plants remove CO₂ from the air. Decay, respiration, and combustion return CO₂ to the air-water reserve. (From *Photosynthesis in Plants*, J. Franck and W. E. Loomis. Courtesy of Iowa State College Press, 1949.)

Carbohydrate transformation and utilization. Cellulose, starch, glucose, fructose, and pentoses, as well as sucrose, occur in most plants. These carbohydrates must be formed from sucrose, glucose phosphates, fructose phosphates, or other intermediate products resulting from photosynthesis. In common with other reactions in living material the reactions by which these products are formed are undoubtedly controlled and regulated by the action of enzymes.

The storage forms of carbohydrates, such as starch and other polysaccharides, must be broken down to monosaccharides or their derivatives before they can be utilized as sources of energy. A similar transformation of insoluble to soluble compounds must be accomplished before the carbohydrates can be translocated from one plant organ to another. The processes of hydrolysis and phosphorylation by which the larger carbohydrate molecules are changed to smaller ones has been discussed in the chapter on seed germination.

Investigations have shown that most of the enzymes required

for carbohydrate metabolism, as postulated in Chapter 8, are present in higher plants. For example, plants have been found to contain phosphorylases, dehydrogenases, decarboxylases, oxidases, and hydrolytic enzymes such as sucrase and amylase. The presence of phosphorylases, dehydrogenases, and oxidases indicates that the oxidation of carbohydrates, with the consequent release of energy, follows a pathway in plants which is similar to that found in other organisms. That is, in the preliminary stages carbohydrates are phosphorylated and changed to triose phosphates. These in turn are converted to pyruvic acid which, through a series of dehydrogenations and decarboxylations, is finally oxidized to carbon dioxide and water. The probable existence of this metabolic pathway is substantiated by the presence of certain organic acids in plants.

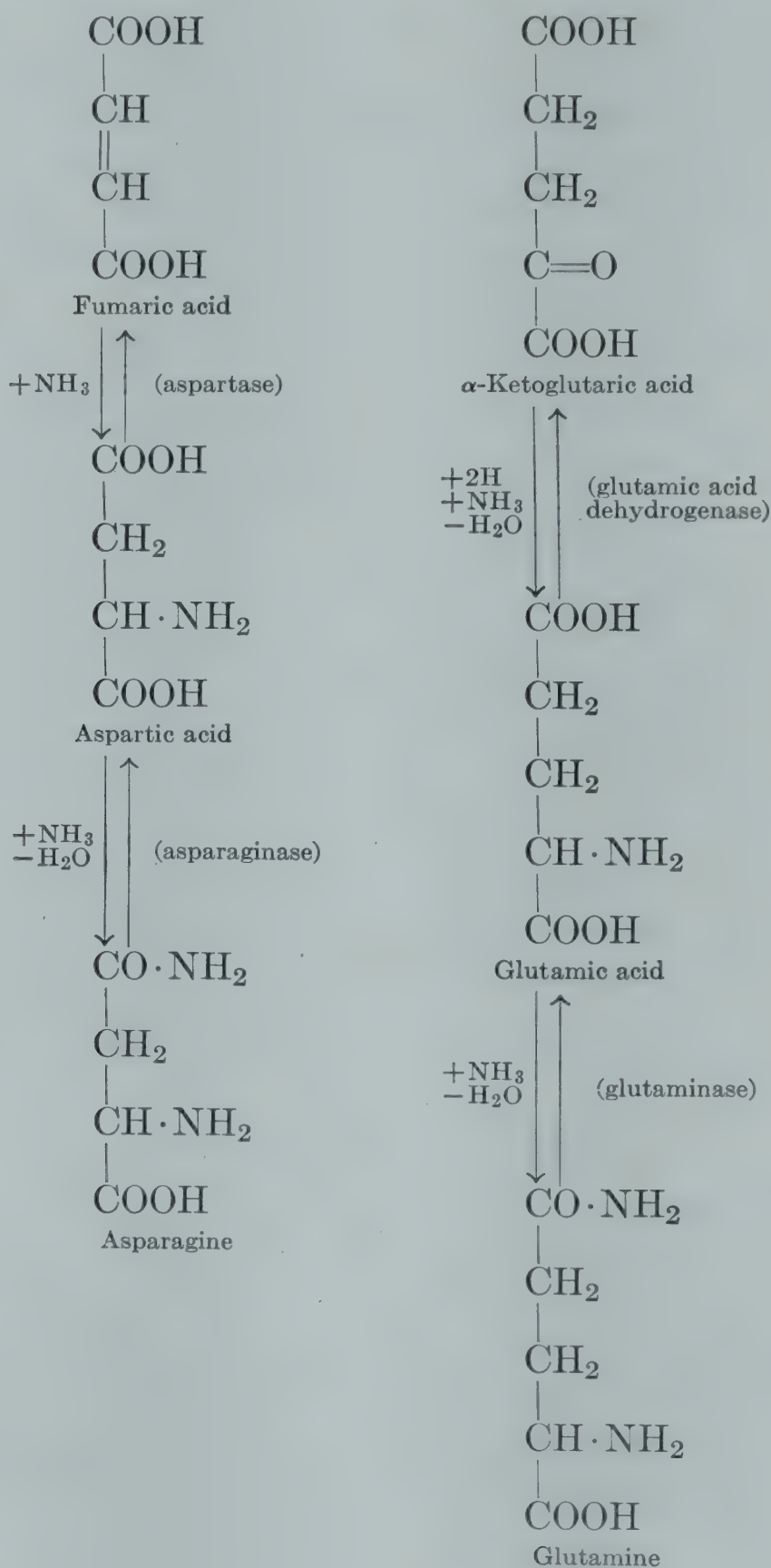
It is well known that fruits such as apples, citrus fruits, raspberries, and grapes contain malic, citric, isocitric, tartaric, and other organic acids. These acids also are present in leaves of plants. Investigations have shown that detached leaves absorb and transform citric, isocitric, malic, succinic, and fumaric acids into other organic acids. A leaf supplied with large amounts of one of these acids converts it into one or more of the acids found in the Krebs tricarboxylic acid cycle. The presence of the enzymes mentioned in the previous paragraph and the ability of plants to utilize organic acids of the Krebs cycle support the hypothesis that a metabolic cycle similar to, if not identical with, the Krebs carboxylic acid cycle is present in higher plants. However, other metabolic systems are undoubtedly present, for no connection has been found between the production or utilization of tartaric and oxalic acids and the Krebs cycle acids.

PROTEIN METABOLISM

During the early phases of seed germination, the principal reactions of proteins are hydrolytic. These catabolic changes take place to some extent during later growth phases but are not so dominant as the synthetic reactions. The anabolic reactions taking place during plant growth produce proteins from inorganic nitrogen sources, organic nitrogen compounds, and other soluble compounds. The principal soluble nitrogen com-

pounds which are possible precursors of proteins are amino acids and the two acid amides, asparagine and glutamine. The syntheses of these protein precursors are accomplished from inorganic nitrogen and products of carbohydrate metabolism.

Asparagine and glutamine can be formed by the following reactions. It should be remembered that these reactions are reversible.



Amino acid formation. The synthesis of amino acids in plants has been demonstrated by studies with detached roots of peas, tomatoes, and flax. When excised roots are placed in nutrient solutions of inorganic salts, only glucose and certain vitamins are required for the production of amino acids. The roots utilize glucose for the production of the carbon framework of amino acids and to supply energy. Energy produced by the oxidation of glucose is required for the synthesis of amino acids since the reaction is endothermic. Plant roots need no source of organic nitrogen to produce amino acids, for they can utilize nitrate and ammonium ions as their source of nitrogen. However, the nitrate ion must be reduced before it can be utilized in the formation of amino acids. The following sequence of reactions is believed to take place:



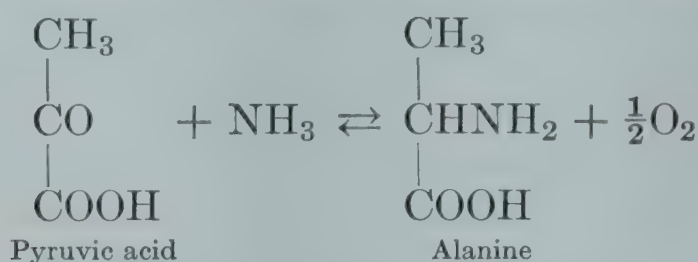
The reduction of nitrates takes place more readily in the leaves of plants than in other plant parts. The fact that nitrate ions must be reduced before utilization does not mean that ammonium ions are the preferred source of nitrogen for plant growth. Many plants thrive better with nitrate than with ammonium ions as their source of nitrogen.

In most plants ammonium ions are rapidly metabolized and do not accumulate in appreciable amounts in healthy tissues. Plants supplied with ammonium ions as their source of nitrogen accumulate amino acids and amides. Ammonium nitrogen supplied at a rate greater than is required for normal growth (luxury supply) may cause carbohydrate depletion. Plants containing more soluble amino acids and amides, but less carbohydrates than normal plants, increase in water content and are said to be more succulent.

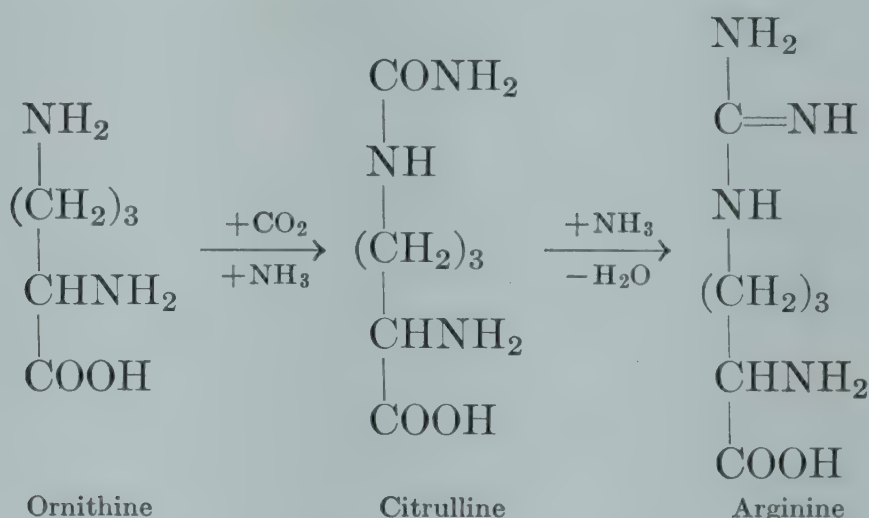
Nitrate-nourished plants, in contrast to ammonium-nourished plants, accumulate nitrate ions and complex organic nitrogen compounds such as polypeptides. A luxury supply of nitrate does not lead to carbohydrate depletion; on the contrary, nitrate-fed plants often show carbohydrate accumulation. The marked contrast in chemical composition between plants nourished with the two common sources of nitrogen indicates a difference in the reactions by which protein is synthesized. The most probable explanation is that nitrates are reduced to the hydroxylamine

stage and that a certain fraction of the hydroxylamine combines with oxaloacetic acid or α -ketoglutaric acid to form oximes. The oximes are subsequently reduced to dicarboxylic amino acids which form polypeptides and proteins.

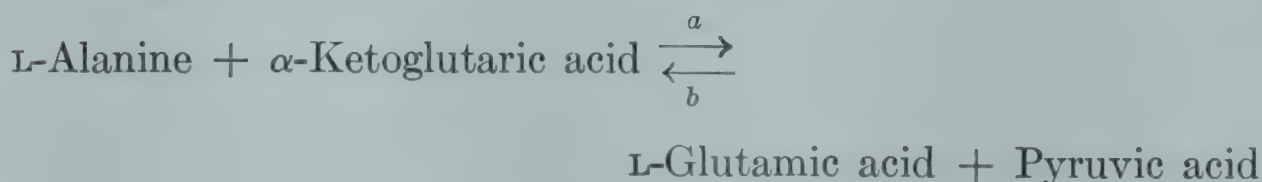
The formation of amino acids from ammonia or ammonium ions is thought to proceed by the reaction of NH_3 with α -keto acids. L-Amino acid oxidases will catalyze such reactions.



Aminases also catalyze reactions by which amino acids can be formed. This was illustrated in a previous paragraph by the addition of ammonia to fumaric acid to form aspartic acid in the presence of aspartase. A third possible method of amino acid formation is illustrated by the formation of arginine from ornithine.



When a supply of certain amino acids has been produced, plants possess a mechanism by which α -amino nitrogen can be transferred from an amino acid to an α -keto acid. This process is called transamination and is controlled by transaminases. These amino transferring agents require pyridoxal as a coenzyme.



Reaction *a* takes place three times as rapidly as reaction *b*. This reaction has been shown to take place in roots as well as in other tissues. The formation of cyclic amino acids such as tryptophan, phenylalanine, and tyrosine must proceed by other methods. Ring closure in the formation of plant compounds is largely unexplained.

Protein formation. The mechanism of protein synthesis from amino acids or other nitrogen compounds is not known. The simplest theory would explain this reaction as a reversal of the hydrolysis of proteins. However, there is little evidence to believe that this is so. There does seem to be a relation between transaminase activity, high concentration of soluble nitrogen compounds (mostly amino compounds), and protein formation. This would indicate that amino acids or closely related compounds are the precursors of protein. The importance of high transaminase activity is unexplained. The hydrolysis of proteins has been discussed in previous chapters. Amino acids are the principal products of protein hydrolysis.

LIPID METABOLISM

Most of the triglycerides found in plants are liquids at room temperature, indicating that unsaturated and short-chain fatty acids predominate. Solid fats are found in tropical seeds such as cacao beans. Plant triglycerides are sometimes arbitrarily differentiated into oils and fats but often are named as one group, the plant or vegetable fats.

Different plant species produce different fats since the ability of a plant to synthesize a given fat is largely an inherited characteristic. However, environmental conditions do affect the composition of the triglycerides produced by a plant species. Cold climates are conducive to the production of highly unsaturated fats. The probable reason for this effect is that the reduction reactions of plants are retarded more by low temperatures than are other reactions responsible for the formation of fats.

Fats are probably present in all plants, although they are not equally distributed among the plant organs. Fats are not found in appreciable amounts in actively growing tissues but

accumulate in storage organs such as seeds and fruits. Various investigations have shown that an increase of fats in maturing seeds is accompanied by a decrease of carbohydrates. The fact that carbohydrates decrease as fats increase has been noted also in excised plant roots and in certain microorganisms which are able to produce triglycerides from media containing glucose as the only source of carbon. These observations indicate a close metabolic relationship between carbohydrates and lipids and support the theory that carbohydrates are the parent material for the production of plant fats.

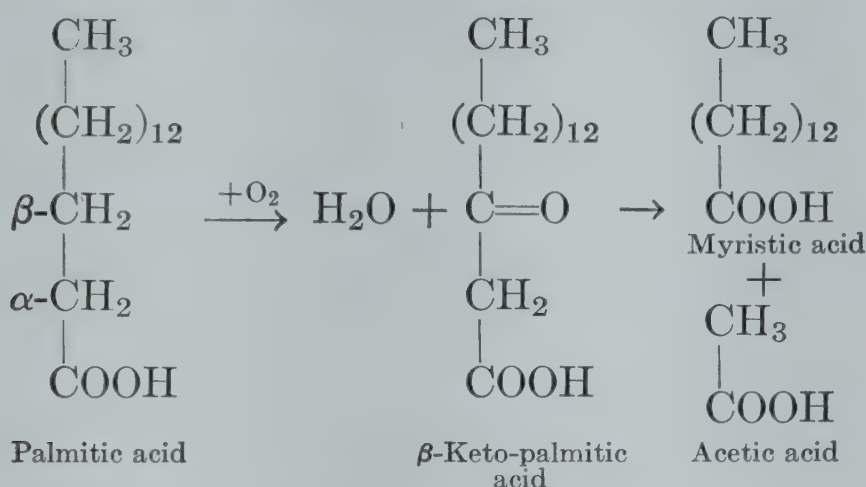
There are probably three principal steps in the formation of fats in plants: (1) the synthesis of fatty acids, (2) the formation of glycerol, and (3) the combination of fatty acids with glycerol to form triglycerides.

Fatty acid synthesis. In general, naturally occurring fatty acids contain even-numbered carbon chains. From this fact it has been postulated that these acids are built up out of a two-carbon compound which may be acetaldehyde or some other derivative of acetic acid. Whether this takes place in plants is uncertain. Although it is known that detached leaves can utilize acetic acid, the products arising from its utilization are unknown. Two-carbon compounds such as acetaldehyde, ethyl alcohol, and acetic acid are produced in carbohydrate metabolism from pyruvic acid. Thus if acetic acid or a derived two-carbon compound is the precursor of a fatty acid we have a metabolic connection between carbohydrates and fats.

Yeast seems to possess a mechanism by which the six-carbon chain of a hexose can be built into fatty acids without passing through a two-carbon stage. Many years before this fact was known Emil Fischer suggested that the condensation of three hexose molecules might take place to form an eighteen-carbon atom chain. The condensation of hexoses would have to be followed by oxidation, by reduction, and probably by dehydration to account for the formation of both saturated and unsaturated acids.

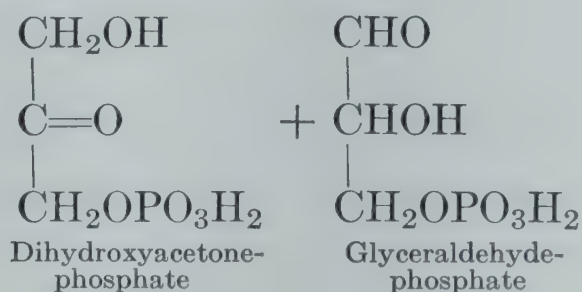
A hexose condensation theory does not explain the formation of C_{14} and C_{16} acids such as myristic and palmitic. Fatty acids which do not contain carbon atoms in multiples of six would

have to be formed by the degradation of longer chains. The removal of two carbon atoms by β oxidation, as suggested by Knoop, would account for the formation of C_{14} and C_{16} acids from the C_{18} compounds. By this hypothesis the carboxyl group and the α -carbon atom are split off and the β -carbon atom is oxidized to the carboxyl group of the new acid.

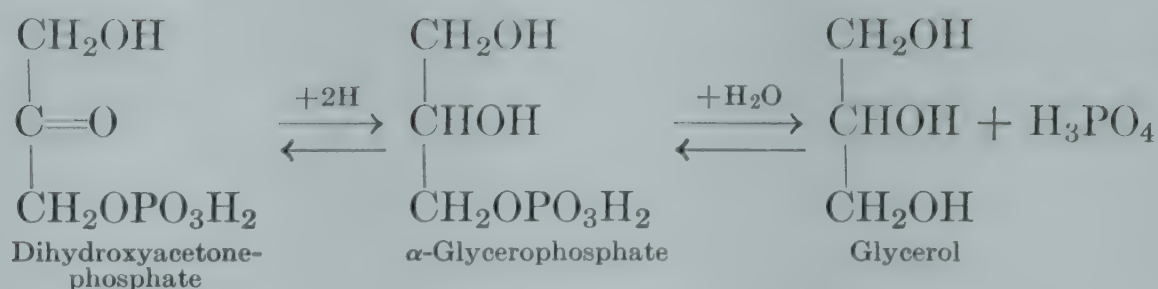


Glycerol formation. Pasteur discovered that glycerol was produced from carbohydrates by fermentation with yeast. More recent studies of fermentation have led to an explanation of the formation of glycerol in this process and to much of our present knowledge concerning the steps in the breakdown of carbohydrates in living cells. The preliminary steps in carbohydrate catabolism, shown in detail in the chapter on biological oxidation, result in the formation of two triose phosphates: dihydroxyacetone phosphate and glyceraldehyde phosphate.

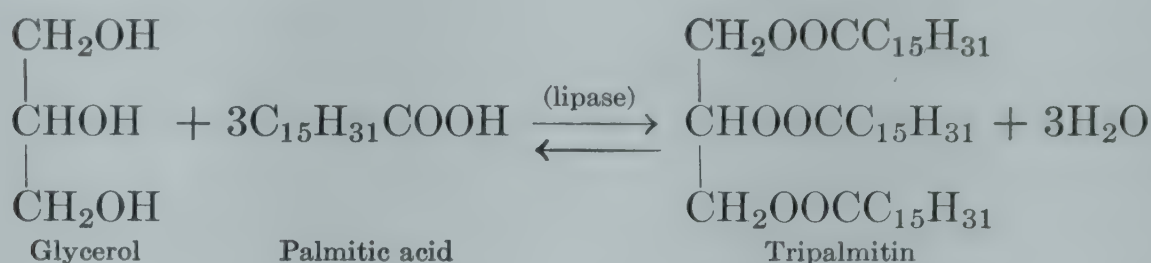
Starch, glucose, or fructose \rightleftharpoons Hexose phosphates \rightleftharpoons



The enzyme α -glycerophosphate dehydrogenase catalyzes the reduction of triose phosphates to α -glycerophosphate. This enzyme has been found in seeds and animal tissues as well as in yeast. Phosphatases hydrolyze α -glycerophosphate to phosphate and glycerol.



Triglyceride formation. Lipase enzymes will catalyze the synthesis and the hydrolysis of triglycerides as illustrated in the following reaction:



The universal presence of lipases in oil-bearing seeds indicates that the final stage in the formation of fats is probably a synthesis of the glyceride from glycerol and fatty acids. A suggested mechanism whereby fats are converted to carbohydrates was given in Chapter 9.

It is obvious that we have discussed only a few of the many reactions that take place in plant metabolism. Many of the compounds known to be present in plants are formed by reactions of which we have little or no knowledge. The subject of plant metabolism is discussed in greater detail in the references listed at the end of this chapter.

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13 · Pesticides

The control of organisms deleterious to plant life is a problem of major importance. Even though the soil and other environmental conditions may be satisfactory, crop production can be impaired considerably by the attack of many harmful insects. In some cases, complete destruction of crops results from insect depredations alone, in spite of the fact that the farmer does all that appears humanly possible in matters of control. The losses to vegetation of all kinds, from these causes, are extremely large each year.

There are many reasons to account for the increased activity of insects in relation to plant destruction. Most of these reasons are probably related to man's changing mode of life over the centuries. A pioneer, for example, cleared just enough woodland to allow him to build a home and have a small garden. It is quite probable that the first plants he cultivated were native to the region. When these plants were freed of competitive vegetation, they grew larger and more fruitful and became more attractive to insects. As more woodland was cleared, and with it the natural vegetation, the cultivated plants became virtually the only source of food for the insects. As communities were developed, and man began his system of intensive cultivation and large-scale farming, the damage to his crops by insects became a serious problem. As his knowledge and experience increased, the farmer gradually accumulated information on various methods of pest control. Among those methods devised for the control of pests, the following are the most important: (1) mechanical, (2) biological, (3) environmental, (4) chemical.

The *mechanical* method involves controlling deleterious insects by (1) building simple traps to capture them or (2) handpicking the insects from the plant. The mechanical method is effective

if the number of plants to be policed is small. The *biological* method of control utilizes the natural antagonism of some insects toward others. The encouragement of the proper insects will result in the destruction of the undesirable species. It must also be remembered that birds, reptiles, and certain small mammals consume large numbers of insects each year. By selecting the proper *environmental* conditions, it is sometimes possible to favor the growth of plants and simultaneously retard the multiplication of those insects which attack them. Moreover, progress has been made recently in developing strains of plants that are resistant to insects.

Although the mechanical, biological, and environmental methods are of interest, the *chemical* method of pest control is the one most frequently employed. As a result the remainder of our discussion will be limited to those substances commonly used for this purpose.

It is difficult to classify the large number of chemicals used for the control of pests. A satisfactory, though simple, classification can be developed, based upon the specific action of the substance as shown in the following outline:

- A. Fungicides—chemicals for the control of fungi.
- B. Herbicides—chemicals for the control of weeds.
- C. Insecticides—chemicals for the control of insects.
 - 1. Stomach poisons.
 - 2. Contact poisons.
 - 3. Fumigants.

FUNGICIDES

Fungicides are preparations to destroy or prevent the growth of fungi. Ordinarily, however, they are a means of prevention and may be applied as dusts or as sprays. By far the most common fungicidal materials are compounds of sulfur and copper.

Copper compounds. Various copper compounds are used as fungicides. The best known of these is *Bordeaux mixture*, prepared by mixing equal quantities of copper sulfate and hydrated lime in the presence of water. In this country, a 4-4-50 Bordeaux mixture is most commonly used. This formulation implies

4 pounds each of copper sulfate and hydrated lime made up to 50 gallons of spray with water. Under these conditions, a voluminous, light-blue, gelatinous precipitate is formed, which remains as a colloidal suspension for several hours.

Bordeaux mixture adheres to plant tissues tenaciously. Sometimes, however, the mixture becomes harmful to plants, owing to the phytocidal action of free copper. The origin of free copper is still in doubt, although several theories have been advanced to explain its presence. Copper injury may be recognized by the presence of brown spots on the tissues or by defoliation. On fruits, the death of some of the cells induces the formation of cork cells, thus causing the russet areas so frequently observed on apples after spraying.

Although Bordeaux mixture is generally conceded to be the best copper-containing fungicide, many other compounds of copper have been tested and used. Among the more successful of these may be mentioned copper ammonium silicate, copper zeolite, copper naphthenate, and copper oleate.

Sulfur compounds. Sulfur has been recognized as an efficient fungicide for more than a century. The more finely it is divided, the more effective it becomes and the better it adheres to vegetation. Sulfur is somewhat volatile under ordinary conditions, and it has been postulated that sulfur vapor is chemically reduced within the spores to form a toxic substance, hydrogen sulfide. Thus, it appears that the fungicidal activity of sulfur may be due to the formation of hydrogen sulfide.

Most of the sulfur used as a fungicide is applied as some form of the element rather than as a sulfur compound. "Colloidal" and flotation sulfur are two popular commercially available forms of the element.

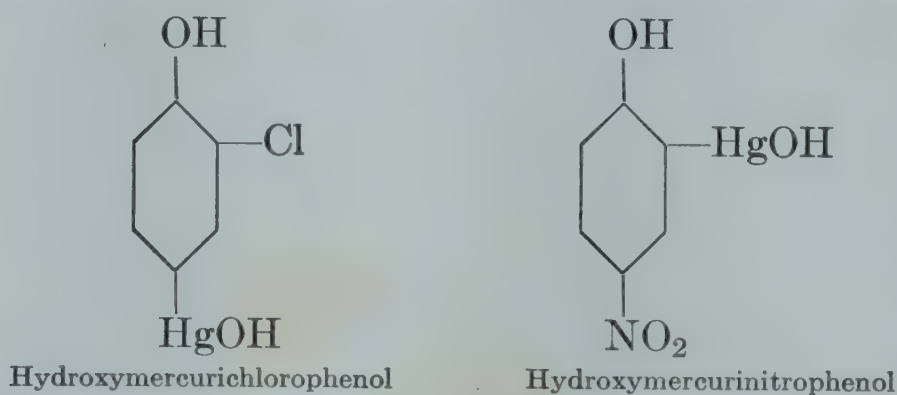
When lime and sulfur are boiled together, various compounds are formed, depending upon the ratio of lime to sulfur, and the temperature of the reaction. Of the numerous compounds formed during the reaction, it appears that the polysulfide compounds possess the greatest fungicidal activity.

Mercury compounds. Both inorganic and organic mercury compounds are used as fungicides, in spite of the fact that these compounds are toxic to all forms of life. Since such compounds are effective in minute quantities, and since they are rarely

used for food plants, the handicap of their toxicity is largely eliminated.

Mercuric chloride (HgCl_2), also known as corrosive sublimate or bichloride of mercury, has found extensive use as a means for the control of scab in seed potatoes. It is also used to control root maggots and other insects and to prevent or eradicate fungus diseases of turf grasses, such as are found on golf greens. Mercuric chloride is extremely toxic to virtually all living organisms. For this reason, it is replaced, whenever possible, by *mercurous chloride* (*calomel*) (Hg_2Cl_2), which is not so toxic to animals as the *mercuric* salt. Whereas mercuric chloride is fairly soluble in water (about 70 grams per liter at 20°C), calomel is nearly insoluble (0.002 gram per liter at 18°C). Calomel may be used alone or in combination with mercuric chloride for the control of cabbage maggots or as a turf fungicide.

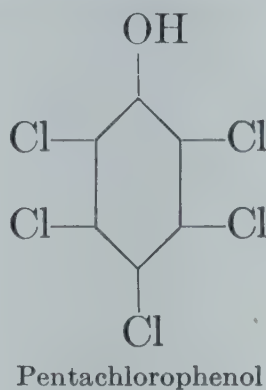
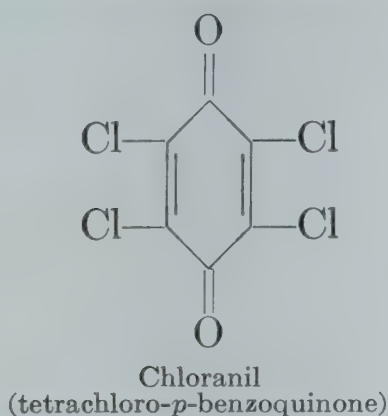
Many mercury-containing organic compounds have been synthesized and studied in the hope that some of these derivatives would be specifically toxic for lower organisms. A number of such compounds are now being used as fungicides for the treatment of seeds. Among others, ethyl mercuric chloride, ethyl mercuric iodide, ethyl mercuric phosphate, hydroxymercurichlorophenol, and hydroxymercurinitrophenol are used for the treatment of seeds. One or more of these compounds serve as the active ingredient of such commercial products as Ceresan, New Improved Ceresan, Semesan, Nu-Green, and Special Semesan.



Organic fungicides. There are a number of organic compounds other than those mentioned in the preceding paragraph that are used as fungicides. Perhaps the best known and oldest of such compounds is *formaldehyde* (HCHO). This simple organic compound has been used for many years as a seed and soil dis-

infectant. Formaldehyde is applied normally in liquid form, although various dry preparations of this compound are available, in which formaldehyde is absorbed upon an inert material, from which it volatilizes slowly on exposure to air.

A number of other organic compounds have been used as fungicides, among which may be mentioned chloranil, carboxylic acids, alcohols and phenols, and pentachlorophenols.



HERBICIDES

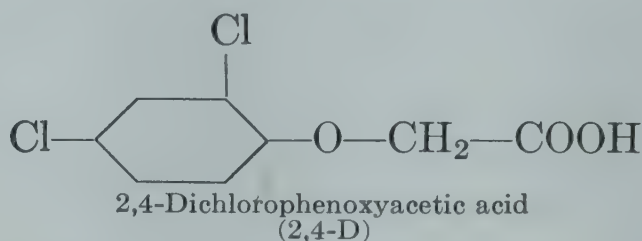
Herbicides are chemical substances used to destroy those obnoxious plants called weeds. Such weed-killers can be classified as *non-selective* or *selective* herbicides. Non-selective herbicides are those chemical substances which are toxic to all forms of plant life, whereas selective herbicides are toxic to the undesirable plants but are not particularly harmful to the desirable species. Although it is relatively simple to destroy all forms of plant life, it is far more difficult to formulate a herbicide that is specific for the destruction of a certain species. Such selective destruction depends upon a number of characteristics possessed by the plants in question. Among these characteristics may be mentioned the size and shape of the leaf, the type of leaf surface (smooth or rough), the susceptibility of the plant to specific chemicals, the toxic threshold of the plant to these chemicals, as well as many other physiological or physical properties of the plant. In many instances it is possible to convert a non-selective to a selective herbicide merely by changing the concentration of the active (toxic) ingredient.

A number of compounds containing *arsenic* have been used as herbicides. Among those compounds that have proved effec-

tive as arsenical herbicides are lead arsenate (PbHAsO_4), calcium arsenate (CaHAsO_4), sodium arsenite (Na_3AsO_3), and arsenic pentoxide (As_2O_5). Arsenic pentoxide is a non-selective herbicide unless the concentration of this substance is carefully controlled. Other arsenic compounds which have been used as herbicides include the sulfoarsenates, the sulfoarsenites, the sulfoxyarsenates, and the pyrosulfoarsenates.

Certain compounds of *boron* appear to have herbicidal properties. It has been found that high concentrations of boron in the soil result in the destruction of a great number of plants. *Sodium chlorate* (NaClO_3) has also been proved an effective herbicide. This compound has disadvantages however, in that it possesses explosive properties and, when mixed with organic matter, becomes spontaneously combustible. Among other inorganic herbicides that have been studied and that are used to some extent as such are copper nitrate, ferrous sulfate, sodium chloride, sulfuric acid, and ammonium sulfamate.

By far the most important herbicide discovered in recent years is the organic compound, 2,4-dichlorophenoxyacetic acid, better known as 2,4-D. This compound is highly toxic to most broad-



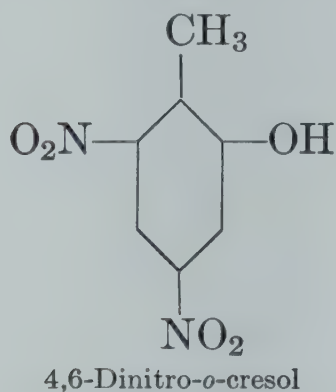
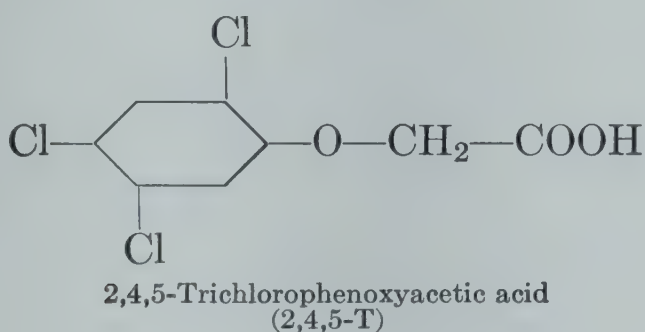
leafed plants, but it is relatively non-toxic to plants of the monocotyledonous type. The free acid is rarely used because of its insolubility in water. However, for the sake of comparison, the other forms of 2,4-D are expressed in terms of the free acid. The most important forms of 2,4-D are the sodium and amine salts and the esters. The ethyl-, isopropyl-, and ethanolamine salts are currently available on the market. Since all these compounds are soluble in water, it is possible to make concentrated sprays from them. Like the sodium salt of 2,4-D, the amine salts are not volatile and therefore will not cause injury to plants unless they come in contact with them.

Ester forms of 2,4-D are also available. These esters are volatile, however, and may cause injury to plants at some dis-

tance, even though the plants are not wetted by the spray. Since the esters are water-insoluble liquids, the commercial preparations of these esters contain an emulsifying agent so that the mixture will be miscible with water in all proportions.

At the present time 2,4-D is used for the eradication of a wide variety of weeds. Since most of the grasses and cereals are comparatively resistant to the herbicide, it has found popular use in the control of weeds in lawns, pastures, and grain fields.

Other organic compounds have been used as herbicides with varying degrees of success. Among these may be mentioned 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and various dinitro



compounds. 2,4,5-T, which is closely related to 2,4-D, has been found useful in killing resistant woody plants. The dinitro compounds are organic materials which in high concentration become virtually non-selective. When properly formulated such compounds find a limited use as selective herbicides.

INSECTICIDES

Insecticides are classified according to their specific action on insects. For example, those poisons which are ingested by the insect and exert their toxic effects in this way are called *stomach poisons*. Some insecticides will kill an insect on contact. Such substances are referred to as *contact poisons*. When an

insecticide acts in the gaseous state, it is called a *fumigant*. In many instances an insecticide is not specific in its action. That is, it may act as a stomach and a contact poison, or as a contact poison and a fumigant. Since the mode of action of different insecticides varies, depending on the species of insect upon which it is to work, one can see that this method of classification is not entirely satisfactory. Consequently, in the following discussion of these substances, the authors have made no effort to divide the compounds according to their mode of action. In each case, however, the mode of action for the poison will be mentioned, if this is possible and if its action is clear.

Inorganic insecticides

Arsenic compounds. In general, insects feed either on the solid portions of the plant or on its sap. Stomach poisons function for the first group but not necessarily for the second. A large number of poisons employed in the control of "chewing" insects contain arsenic in one form or another. Among the first arsenicals to be used for such purposes was arsenic trioxide (As_2O_3). This substance, also called *white arsenic* and *ratsbane*, is slightly soluble in water but more so in carbonated water. White arsenic is extremely toxic to all forms of plant life and consequently finds its principal use in the form of baits.

Calcium arsenate (CaHAsO_4) was introduced as an insecticide about the year 1906. This compound has a higher arsenic content and is cheaper to produce than lead arsenate, but it has several disadvantages which limit its use as an insecticide. Calcium arsenate, when used alone, is relatively unstable, and will cause serious injury to plants. It may be used in conjunction with an excess of lime, in which case the toxicity is decreased. This insecticide has found its principal use as a dust on those plants such as cotton, which are not highly susceptible to arsenical injury.

London purple, a product of indefinite composition, was first employed in the United States in 1878 as a substitute for Paris green in the control of the Colorado potato beetle. It is obtained as a waste product in the aniline dye industry and is composed primarily of calcium and arsenic. It is relatively soluble and tends to "burn" foliage severely. Because of its indefinite com-

position it is difficult to predict the action of this material from batch to batch. Consequently its use has declined rapidly in favor of the more uniform arsenicals, such as lead arsenate.

Paris green, $(\text{CH}_3\text{COO})_2\text{Cu} \cdot 3\text{Cu}(\text{AsO}_2)_2$, is a complex compound containing copper acetate and copper metarsenite. It was the first arsenical to be employed as a spray, having been utilized prior to 1868 for the control of the Colorado potato beetle. Paris green is a heavy substance and does not remain long in suspension. This feature is objectionable as the result is unequal distribution of the insecticide over the plant. At best it does not adhere readily to foliage, making frequent sprayings necessary. Moreover, it contains relatively large amounts of soluble arsenic and will, therefore, cause severe burning of foliage. All these poor qualities have led to the general discontinuance of this insecticide in favor of the more superior substance, lead arsenate.

Magnesium arsenates are compounds that closely resemble the calcium arsenates. These magnesium arsenicals are employed for the eradication of specific pests such as the Mexican bean beetle. This insect cannot be controlled with either the calcium or the lead arsenates, since these compounds do not seem to be toxic to the beetle. Magnesium arsenate, however, will control this insect without apparent injury to the plant.

Lead arsenates. Two forms of lead arsenate are used as insecticides. These are *acid lead arsenate* (PbHAsO_4), and *basic lead arsenate*, which has a probable formula of $\text{Pb}_4(\text{PbOH})(\text{AsO}_4)_3 \cdot \text{H}_2\text{O}$. Of the two, the acid salt is the more commonly used form. In general when one speaks of lead arsenate, the reference is to the acid salt. Lead arsenate is a nearly ideal insecticide. It is easily produced in a form that is readily dispersed in water. Upon spraying, lead arsenate forms a remarkably uniform coating on the plant surfaces and has adhesive properties which leave little to be desired. It does not decompose readily and therefore will not cause injury to the plant as quickly as any of the other insecticides containing arsenic. In spite of the fact that lead arsenate has all these advantages, it must be pointed out that it also has some disadvantages. Among these may be mentioned the fact that lead arsenate is toxic to all forms of life. This is particularly true when we consider that not only the arsenic is toxic to animals, but also the lead, which acts as a cumulative poison in the animal body.

It is quite common to use lead arsenate in conjunction with lime sulfur as both an insecticide and a fungicide. When a mixture of these two materials is made, it is found that injury to plant tissues is increased, probably owing to a release of some form of soluble arsenic resulting from the interaction of the lime sulfur with lead arsenate. In such a case, the addition of lime to the mixture will decrease the formation of soluble arsenic and consequently result in a decrease in injury to foliage.

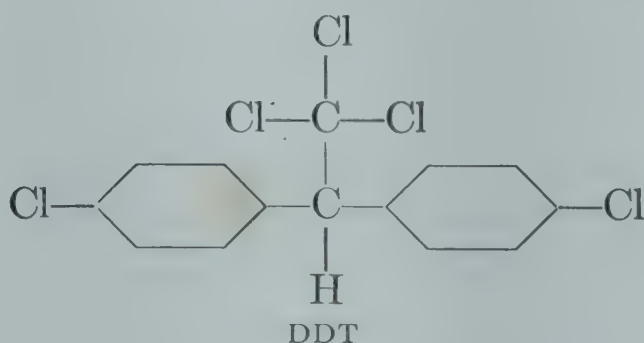
In general it is thought that the basic form of lead arsenate is safer to apply to tender foliage. It must be remembered, however, that basic lead arsenate possesses a lower arsenic content than the acid form and therefore has a lower toxicity, which makes it inferior to the acid salt as an insecticide.

Fluorine compounds. There are a number of compounds of fluorine which have been used as insecticides. Among these may be mentioned sodium fluoride and the fluosilicates of sodium, potassium, calcium, and magnesium. Superior to any of these, however, is *sodium fluoaluminate*, also known as cryolite ($\text{Na}_3\text{-AlF}_6$). Cryolite may be used as a spray or as a dust and is effective against a variety of insects. In general this insecticide has been found to produce very little plant injury.

Organic insecticides

A large number of organic compounds have been examined for their insecticidal properties. A few of those tested have proved of value for the control of insects. Some of the more important organic insecticides will be considered in the following paragraphs.

DDT, 2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane, has become one of the most popular insecticides ever developed. The structural formula for this compound may be written as follows:



DDT possesses many of the characteristics attributed to an ideal insecticide. Acting both as a stomach and a contact poison, DDT exhibits toxic properties toward many species of insects. Moreover this compound can be applied in a number of different ways, either as a dust or in spray form. Accordingly it is marketed as (1) solutions, (2) wettable powders, (3) emulsifiable concentrates, (4) dusts, and (5) aerosols. When applied properly most of the forms mentioned above exhibit considerable residual action.

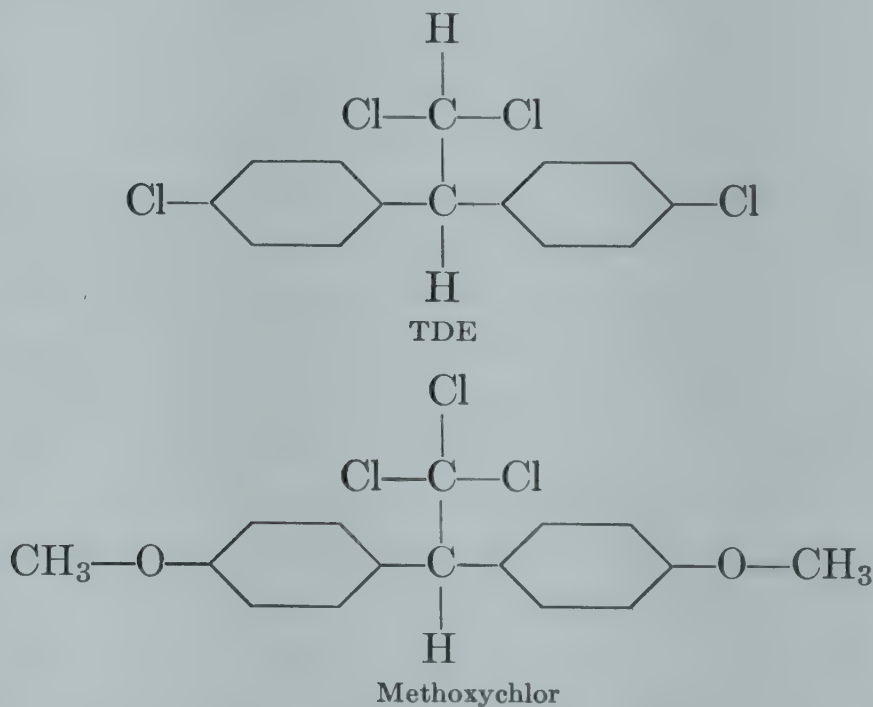
Solutions of DDT are usually composed of 5 per cent of this material in a highly refined petroleum oil. Such solutions may be used for the control of household insects. For this purpose DDT excels because of its unusually high residual action. It has been found that woodwork and walls that have been sprayed with DDT contain small deposits of the insecticide which are effective contact poisons for some time after application. Oil solutions of DDT have been shown to be toxic to animals; consequently care should be taken so that animals or plants are not directly exposed to such sprays. It has been shown that oil solutions of DDT are absorbed through the skin of the animal. Since this substance is cumulative in the animal, it may eventually cause death. For this reason animals or edible crops should not be subjected to large applications of any of the forms of DDT.

DDT supplied as a *wettable powder* may be suspended in water. Such sprays can be utilized to advantage on plants and animals alike, with the exception of dairy cattle, owing to the fact that DDT is fat-soluble and appears in the milk. When the insecticide is applied in this form, it has been shown that the residual action is comparable to that of the oil solution. *Emulsifiable concentrates* of DDT are liquids which, when diluted with water, form stable emulsions. Such emulsions can be employed for those purposes where large residual action is desired, as in barns. DDT emulsions also may be utilized to advantage on certain resistant crops. DDT *dusts* are used on livestock and on crop plants. This form is also popular as a dust for household pets. The residual action of the dust is not so great as any of those forms mentioned above.

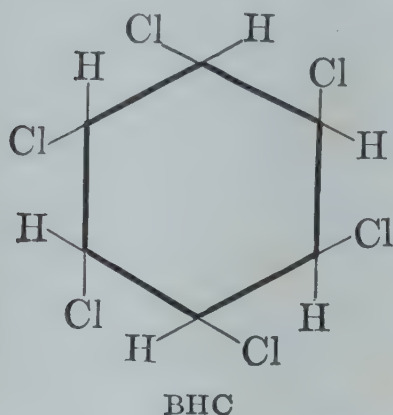
Aerosols are colloidal suspensions of solids or liquids in air. The most common form of aerosol is one in which the colloidal

particles of the toxic material are contained, together with a compressed gas, in a cylinder. When this gas is released, the toxicant is spread in the air and remains in suspension for considerable periods of time. DDT, obtained in aerosol form, is used to a large extent as a household spray for flies, mosquitoes, and various other insects. Because of the relatively low concentration of the active DDT present in the aerosol, the residual action of such a spray is small. Commercial preparations of DDT aerosols usually contain a second insecticide, pyrethrum. The propellant gas in such preparations is usually *Freon-12* (dichlorodifluoromethane).

Many compounds closely related to DDT chemically have been studied with respect to their insecticidal properties. Several of these compounds have been found to be more effective against certain insects than DDT. *TDE*, 1,1-bis-(*p*-chlorophenyl)-2,2-dichloroethane, appears to be effective in the control of the corn borer. Another compound closely related to DDT is *methoxychlor*-2,2-di-*p*-anisyl-1,1,1-trichloroethane. This compound has proved less toxic to higher animals than DDT. The formulas for TDE and methoxychlor are shown below:

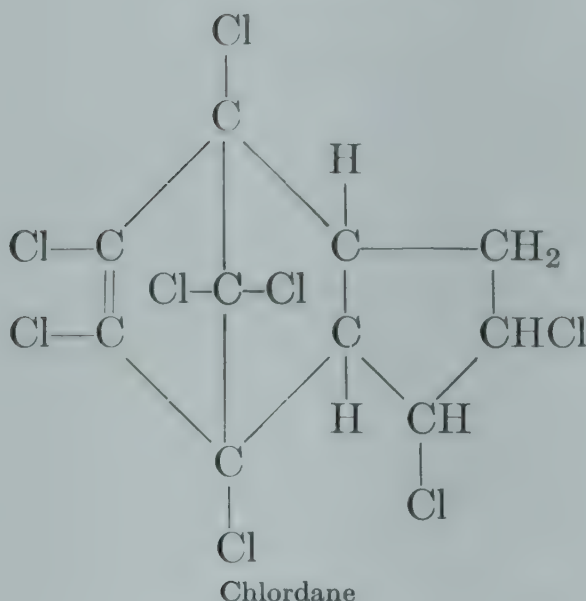


BHC, benzene hexachloride, is another compound that has been recognized as an excellent organic insecticide. The chemical name for BHC is 1,2,3,4,5,6-hexachlorocyclohexane, and its formula may be represented as follows:



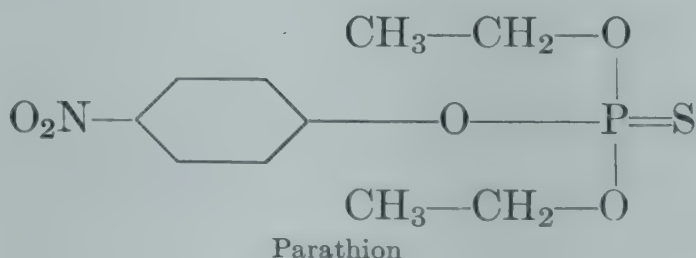
This formula shows that the chemical name used in the literature is incorrect, since it should be named as a derivative of cyclohexane rather than as a derivative of benzene. BHC may exist in sixteen possible isomeric forms. Several of these isomers have been isolated. Of those isolated, the gamma isomer has been shown to possess definite insecticidal properties. This compound is more volatile than DDT and has been proved to be a highly effective insecticide. It has a definite disadvantage, however, in that it imparts a musty odor to the plant. This strong, persistent odor may render the plant unfit for use. Research has shown, however, that, when the gamma isomer of benzene hexachloride is carefully purified, the musty unpleasant odor disappears. Accordingly it has been agreed that, if the gamma isomer is purified to the extent of better than 99 per cent, it shall be designated by the name *lindane*. Lindane, therefore, is the commercial form of extremely pure (99 per cent+) gamma isomer of benzene hexachloride. Lindane, like DDT, has been found to be toxic to animals, and consequently the same precautions must be taken for its use as have been outlined for DDT. In its action toward insects lindane acts as a fumigant, a contact poison, and a stomach poison.

Chlordane is another organic compound that has been used quite successfully as an insecticide. It has been found to possess many of the properties exhibited by DDT. It has a certain amount of residual action, although it is not so persistent in effect as DDT. Chlordane is toxic to animals, and, therefore, care should be exercised when this compound is used on food crops and animals. The chemical name for chlordane, 1,2,4,5,6,7,8,8-octachloro-4,7-methane-3a,4,7,7-tetrahydroindane, may be represented by the following formula:



Several other organic compounds have gained recognition as organic insecticides. Among these should be mentioned the organic phosphates and thiophosphates. These compounds appear to be very effective insecticides, although our knowledge concerning them is far from complete. *Tetraethyl pyrophosphate*, also known as TEPP, has been shown to be effective against such insects as aphids, thrips, and mites. Its action shows that it is both a contact poison and a fumigant. Unfortunately TEPP is toxic to animals, being absorbed through the skin, so that care must be exercised when handling and applying this material. There is not much danger from residues, however, since TEPP is broken down quite readily by the action of water.

Parathion is an organic insecticide chemically related to TEPP. It has many properties similar to the latter compound. Great caution should be taken when handling this material, since it has been shown to be several times more toxic to animals than DDT. It has great possibilities as an insecticide, however, since it is extremely toxic to many insects. The chemical name for parathion is O,O-diethyl-O-*p*-nitrophenyl thiophosphate, the formula for which follows:



Several dinitro compounds have been used as insecticides. Among these are dinitro-*o*-cresol and the dicyclohexylamine salt of 2,4-dinitro-6-cyclohexylphenol. These compounds have been employed as dormant sprays on fruit trees. If applied to actively growing plants, they may cause injury.

Petroleum oils have been used as insecticides for many years. Although crude oils were originally utilized as dormant and summer sprays, the variability of such oils with respect to their impurities made their use difficult. As a result crude oils were replaced by refined lubricating oils, the properties of which vary but slightly from batch to batch. At present *kerosene-type oils* are used in the preparation of cattle and household sprays. In these sprays the oils act as carriers and solvents for such materials as DDT, and other contact insecticides. Kerosene itself is toxic to insects and acts as a secondary toxicant in these sprays. *Light- and medium-grade oils* have been utilized as summer sprays. "Superior-type" oils, composed of paraffinic hydrocarbons, have been shown to be more toxic to insects than oils containing large quantities of naphthenic and aromatic compounds. *Heavy oils*, on the other hand, are referred to as dormant oils. Such oils, which possess a much greater viscosity than the light or medium oils, are used for dormant spraying of shrubs and trees. These oils destroy insect eggs present on and under the bark of trees. Plant injury results if any of these oils are used in excess. It has been shown that plants sprayed with petroleum oils possess lowered transpiration rates and reduced photosynthetic activity.

FUMIGANTS

Fumigants are pesticides which exert their toxic action in the gaseous state. Although such compounds may be applied in solid, liquid, or gaseous form, they must all exhibit the very important property of volatility. Since the toxic action of these compounds depends upon their volatility, it is obvious that fumigants are designed for effective use in closed spaces, such as dwellings and greenhouses.

Although a great number of compounds have been examined with respect to their fumigating action, only a few have been accepted as important fumigants. Among these may be men-

tioned hydrocyanic acid, carbon disulfide, carbon tetrachloride, methyl bromide, ethylene dichloride, naphthalene, and paradichlorobenzene.

Hydrocyanic acid, also known as *hydrogen cyanide* or *prussic acid*, has the formula HCN. This substance is extremely toxic to all forms of animal life. Consequently great care must be observed in handling this material, and applications of prussic acid should be made only by experienced operators. Hydrocyanic acid is particularly useful in the fumigation of dwellings and greenhouses. For this purpose the gas is generated by the action of a mineral acid on a salt such as potassium or sodium cyanide. If warehouses or mills are to be fumigated, the application may be made by the release of the compressed gas which can be stored in cylinders. Since HCN is so toxic, small quantities of irritant gases are sometimes mixed with the fumigant in order to give warning of the presence of the toxic substance. In order to be effective, the irritant must diffuse at nearly the same rate as the toxic material. Chloropicrin (tear gas) and cyanogen chloride are often used for this purpose.

Carbon disulfide (CS_2) is readily volatilized at room temperatures. The vapors of this material are more than 2.5 times as heavy as air. CS_2 has a very disagreeable odor and is highly flammable. Carbon disulfide vapor will explode if it comes in contact with hot surfaces such as steam pipes or hot radiators. Carbon disulfide is extremely toxic to all forms of life. The fact that the vapors of this material will destroy plants and certain seeds limits its use to fumigation of dwellings and warehouses. Carbon disulfide may also be used as a soil insecticide where, owing to its density, it will permeate the soil and destroy insects.

Carbon tetrachloride (CCl_4) is not flammable, making it an extremely safe fumigant. Unfortunately, however, this material is not so toxic to insects as carbon disulfide, and therefore much larger quantities must be used in order to kill the insects. This fact limits the use of carbon tetrachloride to those places where fire hazards prohibit the use of carbon disulfide.

Ethylene dichloride ($\text{C}_2\text{H}_4\text{Cl}_2$) has been used as a fumigant for the control of the peachtree borer, Japanese beetle larvae, and

other organisms. It has also been used effectively as a fumigant when mixed with carbon tetrachloride. Such mixtures may be used by inexperienced operators, since they are relatively non-toxic to humans. Moreover, such mixtures have the added advantage that they are not flammable.

Methyl bromide (CH_3Br) a non-flammable substance, has found wide use as a fumigant. It has been shown that prolonged exposure to this material is injurious to animals. Moreover, repeated short exposures to methyl bromide have been shown to be cumulative, unless several days elapse between such exposures. Methyl bromide is toxic to a large number of insects and is used extensively to control pests in mills, warehouses, freight cars, and ships.

Paradichlorobenzene ($\text{C}_6\text{H}_4\text{Cl}_2$) is commonly referred to as PDB. This substance has found wide use in the control of the common clothes moth. For this purpose PDB may be marketed as a solid cake, in flake or crystal form, or in solutions with which the clothes may be sprayed. PDB is useful also as a soil fumigant for the control of the peachtree borer.

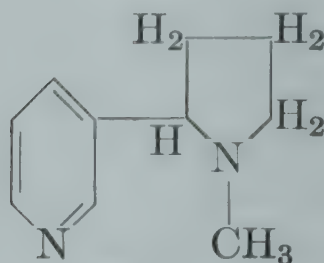
Naphthalene (C_{10}H_8), popularly known as moth balls, has been used as a fumigant for many years. It has been used effectively against the clothes moth and thrips. Naphthalene is relatively toxic to young plants but becomes toxic to animals only in very high concentrations.

INSECTICIDES OBTAINED FROM PLANTS

A number of well-known insecticides are derived from plants. Among these may be mentioned nicotine, rotenone, pyrethrum, ryania, and sabadilla. Most of the insecticides obtained from plants act as contact poisons and are characterized by their high toxicity to insects.

Nicotine has been used as an insecticide for several hundred years. Before this alkaloid was extracted from the tobacco plant as a liquid, the stalks and the leaf midribs of the plants were ground to a powder and applied as a dust.

Nicotine has the chemical name 3-(1-methyl-2-pyrrolidyl)-pyridine and may be represented by the following formula:



Nicotine

When isolated in pure form nicotine is an odorless, water-white liquid. When nicotine is allowed to stand in the presence of light and air, it will rapidly turn dark in color and become thick and syrupy. Free nicotine is extremely toxic to all animals. Consequently nicotine is generally marketed as nicotine sulfate, which is somewhat less toxic to man. Solutions of nicotine sulfate are used to control aphids, in which case the insecticide acts as a contact poison. Nicotine can also be used in greenhouses as a fumigant, in which case tobacco stalks and leaf midribs are burned, liberating the insecticide as a gas.

Rotenone is an insecticide obtained from the roots of certain plants. Among these may be mentioned the genera *Derris*, *Lonchocarpus*, *Tephrosia*, and *Millettia*. Of these the derris root is most commonly used for the isolation of the active insecticide. Rotenone is generally applied as a dust, although some commercial preparations may be used as sprays. Since rotenone is relatively harmless to warm-blooded animals, it is recommended for use on such edible crops as beans. Many louse powders for livestock contain rotenone as one of their active ingredients.

Pyrethrum is a product derived from the flowers of a species of chrysanthemum. The active ingredients of this insecticide have been shown to be chemical compounds designated as *pyrethrins* and *cinerins*. Pyrethrum is relatively harmless to higher animals and may be used in household insect sprays. Pyrethrum possesses little residual action, killing insects mainly on contact. As a result, it has limited usefulness as a spray in enclosed spaces, such as a room or a barn.

Ryania and *sabadilla* are insecticides obtained from plant sources. *Ryania* has been used with success for certain insects, among which is the European corn borer. *Sabadilla* has found use in controlling leaf hoppers, grasshoppers and the gypsy moth.

MISCELLANEOUS PESTICIDES

Rodenticides. The annual crop damage due to rodents is of great significance to the agriculturalist. Accordingly the destruction of rodents becomes a matter of economic importance. Chemical compounds used for the destruction of rodents are called rodenticides. The toxic action of some of these materials is based on the fact that rodents cannot regurgitate food. The ingestion of poisoned food, therefore, will cause death. Some of the more important rodenticides are Red Squill, Antu, phosphorus, sodium fluoacetate, DDT, and strychnine.

Red Squill is prepared from the ground bulb of a plant and contains several alkaloids of unknown composition. The death of the rodent is attributed to the toxic action of these alkaloids. *Antu* is a popular rodenticide, the active ingredient of which is α -naphthylthiourea. *Phosphorus* has also been used for many years as a rodenticide. This is a dangerous poison since it will cause the death of any animal ingesting it. *Sodium fluoacetate* is *extremely toxic* to all animals. For this reason this chemical is not available to the general public. In some instances, grain is coated with the alkaloid, *strychnine*, and placed in or near rodent burrows. Although strychnine is an effective poison, it must be used with caution since it is toxic to all animals. *DDT* has become a popular rodenticide for the control of mice. Rodents that track through this material will, in cleaning their feet, ingest enough of the substance to cause death.

Animal dips and sprays. The problem of removing lice and various insects from cattle and sheep is of major importance, particularly in those areas where large numbers of animals are raised. For many years the standard procedure for the removal of such pests was to subject the animal to a solution of a disinfectant such as creosote. The common cattle and sheep dips of the past were solutions of this material. More recently the animals have been treated with rotenone dusts or DDT sprays. These have been found to be highly effective and in many instances more efficient than the older method of dipping.

SPRAY RESIDUES

The presence of toxic spray residues on fruits and vegetables has become a problem each agriculturalist must recognize. At the time this is written, the law states that each pound of fruit may not contain more than 0.025 grain of arsenic (calculated as arsenic trioxide), 0.05 grain of lead, or 0.02 grain of fluorine. In order that he may adhere to these tolerances each grower has several alternatives from which to choose. They include: (1) application of small amounts of toxic materials with the hope that the crop will be adequately protected; (2) application of materials that are toxic to insects but not to man; (3) no application of insecticide in the hope that the crop will not be damaged by insects, and (4) application of sufficient quantities of toxic material to protect the crop, followed by the removal of toxic residues prior to marketing. The last method is the one most commonly used at the present time.

In addition to lead, arsenic, and fluorine compounds, it is possible that the residues of other pesticides are toxic if such compounds are used in high concentrations or over long periods of time. It should be stressed, therefore, that the agriculturalist should use caution in the application of any insecticide.

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14 • Farm Chemurgy

From the beginning of time man has depended on plant and animal products for food, clothing, and shelter. As civilization advanced he found an increasing number of non-food uses for agricultural products. Nevertheless farm crops never played a major role in modern non-food industries, owing to the fact that they were usually more valuable as foods and feeds.

During the world depression in the late nineteen-twenties and early nineteen-thirties, American farmers were faced with large surpluses of agricultural products for which there were no markets. As a result agricultural and industrial scientists and Government officials became interested in finding ways and means of helping agriculture dispose of these surpluses at prices that would cover cost of production.

In the late nineteen-twenties Dr. William J. Hale of the Dow Chemical Company approached the problem from the standpoint of industry, with the hope that some of the agricultural surpluses could be used for new industrial purposes. Wheeler McMillen, agriculturalist and journalist, became actively interested in the same problem with the objective of stabilizing agriculture by finding new markets for agricultural products. These pioneers literally "stumped" the country in an attempt to make agricultural, industrial, and political leaders aware of the necessity for concerted action for the solution of this important economic problem.

Dr. Hale coined the term *chemurgy*. The term is derived from the Egyptian word *chem* and the Greek word *ergon*, to imply that chemistry was being "put to work." Finally the term *farm chemurgy* was adopted to indicate that chemistry is working for the solution of farm problems.

Hale and McMillen solicited the aid of many prominent gov-

ernmental and industrial leaders, including Herbert Hoover, Henry Ford, and Thomas Alva Edison. As a result of these activities the Farm Chemurgic Council was created in 1935. Later this organization became known as the *National Farm Chemurgic Council*. The Council played a major role in arousing national interest in farm chemurgy. As a result of this educational campaign Congress established four Federal regional research laboratories to investigate farm chemurgic problems. The laboratories, costing approximately two million dollars each, were equipped with personnel and physical facilities for the application of chemistry, physics, biology, and engineering to the solution of various types of problems involving industrial utilization of farm crops.

The Southern Regional Research Laboratory was established at New Orleans to investigate new uses for such products as cotton, peanuts, sweet potatoes, and other crops produced in the south. The Northern Laboratory was placed at Peoria, Illinois, to study wheat, corn, and agricultural wastes. The Eastern Laboratory was built at Wyndmoor, near Philadelphia, Pennsylvania, to investigate the utilization of such products as milk, apples, vegetables, Irish potatoes, and tobacco, and the Western Laboratory at Albany, California, was established to conduct studies on alfalfa, fruits, and vegetables. Many state agricultural experiment stations initiated similar experiments on crops of local interest, and several industrial research organizations established various types of farm chemurgic research projects.

From a chemical standpoint it is conceivable that almost any type of non-metallic industrial product can be made from natural materials. It should be borne in mind, however, that industry, as a rule, cannot afford to pay food prices for natural raw materials to be fabricated into industrial products. This is one of the many economic difficulties that farm chemurgy must recognize. Furthermore no industrialist is willing to invest large sums of money in manufacturing plants for the utilization of crop surpluses when there is no assurance that these surpluses will be available year after year at prices he can afford to pay.

Consequently it would appear that agricultural wastes seem to offer the best opportunity for industrial exploitation, since these residues must be utilized or destroyed. It is difficult, however, to

find some of these wastes concentrated in a small area in sufficient quantities to merit installation of processing plants. Transportation of bulky and perishable farm residues to a central processing point is another economic problem.

Furthermore the creative research chemist is continuously on the alert with regard to possibilities of producing synthetic substitutes, as soon as he is convinced that a potential market exists for these substitutes. Often the organic chemist is in the advantageous position of being able to start with inexpensive raw materials and, by synthetic procedures and mass production methods, to produce a quality product at a price that will drive the more expensive natural product from the market.

Synthetic indigo, for example, has completely replaced the natural dye. This has resulted in the complete abandonment of indigo farming in India. At one time milk whey was the sole source of riboflavin (vitamin B₂), and special factories were built for the extraction of riboflavin from milk whey. Today synthetic riboflavin has completely replaced the natural product and is sold at a price at which the natural product cannot compete.

A few years ago crystalline ascorbic acid (vitamin C) was extracted commercially from Hungarian peppers. At one time crystalline ascorbic acid sold for as much as three hundred dollars a gram. Today the synthetic product is made by the ton and is available in quantity at less than three cents per gram. Many other similar examples could be cited if space permitted.

For these reasons there is considerable economic risk in growing certain types of special crops for the production of special chemicals. At the present time the insecticide industry depends on agriculture for the production of pyrethrum from which are extracted the pyrethrins, toxic substances used for the control of flies and other insects. Government chemists have shown, quite recently, that the pyrethrins can be made synthetically in the laboratory. It is conceivable that the time is not far distant when natural pyrethrin products can no longer compete with the synthetic product.

Nevertheless many phases of farm chemurgy have been and will continue to be economically successful, to the mutual benefit of the farmer, the industrialist, and the ultimate consumer.

It is not possible in this brief chapter to do more than point out some of the more significant phases of farm chemurgy. Space will not permit discussions of the chemistry of the various processes, nor is it possible to describe manufacturing methods. The following discussion is an attempt to give an impartial evaluation of present and future industrial uses for agricultural crops and crop residues.

INDUSTRIAL PRODUCTS MADE FROM FATS AND OILS

Vegetable oils

The most common sources of vegetable oils are the oil seeds such as soybean, cottonseed, linseed, corn, castor bean, and sunflower. Peanut oil is an important nut oil, and some oil is also extracted from almonds, pecans, and similar nuts. Tung oil is obtained from the nuts of the tung tree. Originally China was the principal source of tung oil, an oil highly prized for its drying properties. Tung trees grow well in certain areas of southern states bordering on the Gulf of Mexico. Although tung oil production in the United States has increased in recent years, the amount of domestic tung oil falls far short of the demand.

Approximately 60 per cent of the 5 million tons of oils and fats produced annually in the United States is derived from vegetable sources. Until recently about one-tenth of the total production came from cottonseed. At present soybean oil production exceeds that of cottonseed oil. Nearly three-fourths of the oils and fats produced in the United States are used as foods; about one-fifth finds its way to the soap industry, and the remainder (about 10 per cent) is used for miscellaneous industrial purposes.

Tung and linseed oils are in great demand as drying oils for paints, enamels, and lacquers because they are highly unsaturated. Semidrying oils, such as castor and soybean oils, can be made into drying oils by suitable chemical processing. Rape seed, castor bean, and mustard seed oils are used for special lubricating purposes.

Glycerine, an important by-product of soap making, is used in the manufacture of nitroglycerine, in pharmaceutical preparations,

in cosmetics, and in tobacco products. Free fatty acids are obtained from oils and fats and are used in soap making. They can also be polymerized and used in commercial plastics and coatings. So-called soapless detergents can be made from lauric and palmitic acids, and the polyamide resins, used in the plastics trade, are made from fats or their constituent fatty acids.

Food uses of vegetable oils include shortenings, hydrogenated cooking fats, margarines, and salad oils. Industrial uses for vegetable oils include the manufacture of linoleum, oilcloth, shade cloth, leather goods, and printing inks. In the metallurgical industry these oils are used as core oils and in the rolling of tin plate.

Many commercial emulsions require the presence of vegetable oils. Other commercial outlets for these oils include such industrial products as insecticides, roofing pitches, waxes and floor polishes, cosmetics, shampoos, shaving compounds, adhesives, lubricating greases, rubber, and others too numerous to mention. Tall oil is a by-product of the paper industry. When paper is made from pine wood by the sulfate process, an oil is recovered which has found wide use in the manufacture of linoleum, paint, varnish, oilcloth, and soap.

The Sugar Research Foundation has developed a process for the extraction of a wax, from sugar cane, which can be used as a substitute for the more expensive imported vegetable waxes. This Foundation estimates that the continental United States, Hawaii, Puerto Rico, and Cuba throw away, annually, about 60 million pounds of crude sugar-cane wax. Their technical experts estimate that 38 million pounds of the above-mentioned crude material could be made available as a dark-colored wax suitable for industrial purposes. No use has been found, as yet, for the 22 million pounds of fatty residues remaining after the wax is extracted.

Soybean lecithin is becoming a useful by-product of the soybean industry. Soybean oil contains from 2 to 5 per cent of these phospholipids. Lecithin is used in the food industry as an emulsifying agent and antioxidant and in the confectionary trade to preserve the "bloom" and prevent graying of chocolate in hot weather. Lecithin is also used in compounding cosmetics and

pharmaceutical preparations. This phospholipid is also a constituent of peanut oil and other vegetable oils.

Animal fats

Animal fats have been used primarily as food fats in the form of lard and margarines. In recent years, however, the vegetable margarines have superseded the animal margarines. Since vegetable and animal fats are glycerides of fatty acids, it is to be expected that they can be used interchangeably for many industrial purposes. Animal fats are characterized by a low content of unsaturated fatty acids. Consequently they tend to be solid or semisolid at ordinary temperatures and cannot be used in industrial processes requiring highly unsaturated fatty acids. Nevertheless animal fats find many non-food uses in industry. Stearic and palmitic acids are used in compounding rubber, in lubricants, hard and soft waxes, rainproofing of textiles, package and paper coatings, wetting agents, reclaiming of rubber and in soap making.

Fish oils also play an important role in industry. With proper chemical treatment and purification they can be used for many of the industrial processes described for vegetable and animal fats. Much of the crude fish oil is used for soap making. Body and liver oils of marine fish are used for domestic animal feeding. Liver oils, from such fish as the cod and shark, are in great demand as sources of vitamins A and D. These and other potent fish-liver oils are sold in enormous quantities by pharmaceutical houses for human consumption and by other commercial groups for supplementing poultry rations.

INDUSTRIAL PRODUCTS MADE FROM CARBOHYDRATES

Agricultural crops which are valuable sources of carbohydrates may be divided into three classes: (1) crops rich in the natural sugars, (2) crops containing commercial amounts of starches, and (3) crops valued for their content of cellulose and related polysaccharides.

The natural sugars

Sucrose. This sugar is, of course, of great chemurgic importance. It is manufactured from sugar cane and from sugar beets. Sugar cane is grown in greatest quantity in Cuba, Puerto Rico, and Hawaii and to less extent in Louisiana and Florida. Sugar beets are produced in largest volume in irrigated sections of the western United States, particularly in the state of Utah. The principal uses for sucrose are as foods and candy products, and large amounts are dispensed in the form of molasses in certain types of livestock feeds.

The principal non-food products derived from sucrose are chemicals produced by fermentation processes. The most important fermentation product is, of course, ethyl alcohol, which is made from molasses. In sugar-producing areas rum may be the principal product of molasses fermentation. Ethyl alcohol is an important industrial product, not only because of its solvent properties, but also because it is an important starting chemical for the synthesis of literally hundreds of chemical compounds.

The Sugar Research Foundation has listed 115 chemical compounds that can be formed from sugars by the action of microorganisms (yeasts, molds, and bacteria). Representative industrial fermentation products made from sucrose are citric acid, lactic acid, various alcohols, and levulinic acid. The last-mentioned acid is of interest to the organic chemist because it serves as a starting material for the synthesis of heterocyclic compounds. Lactic acid may be polymerized to form a plastic which can be molded to form many useful articles. Sucrose can be hydrolyzed to form invert sugar in which form it is used as fondant in candy making.

Other natural sugars. Other natural sugars meriting mention are lactose (from milk) and pentose sugars which occur as pentosans in woody and fibrous crops. The latter will be discussed with the celluloses. Lactose, a by-product of the dairy industry, remains in the whey after butter fat and casein are removed. Milk sugar is marketed as a white crystalline powder and is used almost entirely for compounding pharmaceutical preparations and in infant and invalid foods.

Apple syrup or apple honey is made by concentrating apple cider or the washings from apple pomace. It has found limited use as a flavoring material in tobaccos and as a humectant (moisture retainer) for tobacco products on account of the high sugar content.

Starches

Crops that are valuable sources of commercial starch are the cereals (corn, rice, and wheat) and the tubers (Irish and sweet potatoes). Of these corn is the most important because it is produced in greater quantities than any other American cereal. Although approximately 3 billion bushels of corn are produced annually in the United States, only about 3 per cent of this amount finds its way into non-food industrial channels. The remaining 97 per cent is used as food for human beings and domestic livestock.

Much of the corn utilized industrially is used for starch manufacture, although appreciable quantities are purchased by the fermentation industries for the manufacture of alcoholic beverages and industrial alcohol. Until comparatively recent years tapioca starch was imported by thousands of tons because the presence of amylopectin gives this starch desirable physical characteristics. Cornstarch, which is characterized by less amylopectin and more amylose, cannot compete with tapioca starch for certain purposes. In recent years new varieties of waxy maize and waxy sorghum have been developed, the starches of which contain the desired proportion of amylopectin. Many technical workers feel that these new starches will be able to replace completely the imported tapioca starch.

Prior to the wet milling of corn for starch making the corn is steeped in water containing SO_2 to remove undesirable microorganisms and to assist in the degradation of the hard corn kernel. The acid-water solution is drawn off and is known as *steep liquor*. Products of corn processing are numerous. These include corn oil (obtained from the embryo), starch, corn syrup, corn sugar, dextrans, and proteins. These do not include the corn residues, gluten feed, and steep liquor. The latter is concentrated and added to livestock feeds. Some steep liquor is sold as a nutrient

medium for the production of penicillin. It is also used as a nutrient medium for production of commercial yeast. The wet milling industry supplies several other industries with corn products. Examples of these are food processing, paper, textile, adhesive, chemical, and pharmaceutical industries.

Starch is used in enormous quantities for laundering, for sizing of textiles, and for manufacture of adhesives. Corn sugar is used in the food and confectionary industries and in the chemical industry for the manufacture of mannitol and sorbitol. The latter is the chief base material for the manufacture of synthetic ascorbic acid (vitamin C). About 200 products are said to be made from corn.

Starch is also made from potatoes. Late in the last century there were approximately 150 potato-starch factories in the United States. The production of cornstarch began about 1880, and the production of potato starch decreased, largely because of potato storage, transportation, and spoilage problems. Nevertheless the potato-starch industry is firmly established, in spite of low production and the higher price of potato starch. Relatively small plants are situated in potato-growing sections of the United States where an abundant supply of good water is available.

Potato starch is used principally in the textile industry for the sizing of cotton, spun rayon, and worsted warps. It is also in demand as a source of adhesives, in paper sizing, and in fine laundering. The chemical and physical properties of potato starch are quite similar to those of cornstarch, although the former is preferred to the latter for some industrial purposes.

Important chemicals that can be made from starches (starch sugars) include organic acids, esters, acetone, ethyl alcohol, and butyl alcohol. During World War II butyl alcohol was used for the manufacture of butadiene, which formed synthetic rubber on polymerization.

There has been considerable interest in the blending of grain alcohol with gasoline to extend our gasoline supplies. Although the idea is feasible, there are, as yet, too many economic and other barriers to make the project successful at the present time. Alcohol-gasoline blends have been used successfully in Europe where alcohol is relatively abundant and motor fuel is scarce.

Commercial utilization of cellulose crops

Agricultural sources of cellulose include cotton, wood, straw, cornstalks, corncobs, oat hulls, rice hulls, bagasse (sugar cane residues), hemp, flax, jute, and ramie. The types of commercial products made from cellulose may be classified as follows: (1) fiber products, (2) textiles, and (3) chemicals. All these products owe their physical and chemical properties to cellulose or, in a few cases, to associated pentosans.

Fiber products

Industrial materials in this group include such products as paper pulp, cardboard, paper, wallboard, and insulating materials.

Wood. Paper pulp and paper are made almost entirely from forest trees, although excellent grades of paper can be made from many fibrous plant materials, including straw and cornstalks. Cornstalks have not been used commercially for the reason that the raw materials are scattered over large areas and transportation costs are excessive. Recent advances in the production of rapidly growing slash pine trees in the south have been a great stimulus to the southern pulp and paper industry. Slash pine can be grown to commercial size in about 10 years as compared with 40 and 50 years for the northern spruce.

Wood residues are also utilized for industrial purposes. It is estimated that wood waste totals more than 109 million tons annually and that about 40 per cent of this is used as fuel. Lignin and chemical wastes from the pulp and paper industry are estimated at 8.6 million tons. These wastes are being used to some extent as insulation fillers, adhesive and plastic fillers, rolled roofing fillers, building felts, wallboards, and composition shingles. Some sawdust and shavings find a market as wood briquettes for fuel. Chemical products made from wood will be discussed later.

Lignin, until recently a useless by-product of the paper mills, is beginning to find industrial uses. During pulping, wood particles are treated with sulfite or soda liquor and heated under

pressure. The cellulose fibers are freed from extraneous materials and are made into pulp and paper. The residual lignin may be treated with phenol to form plastic adhesives; these adhesives are being used as a bonding substance for plywood veneer, as corrosion inhibitors, as flotation agents in mining, and as ion-exchange resins for water softening.

Cellulose is also used for the manufacture of artificial silk (rayon). Viscose silk fibers are made by soaking cellulose in sodium hydroxide, and the gelatinous mass is caused to form a heavy yellow solution by adding carbon disulfide. After aging, the solution is pressed through small orifices to form plastic threads which are solidified or precipitated by passing into acid solution. These threads have an attractive sheen and good tensile strength and are woven into rayon fabrics.

Cotton. From a strictly farm chemurgic standpoint, cotton is the most important fiber crop. About 11 million bales of this almost pure cellulose fiber are produced annually. Cotton's use as a textile fiber will be discussed later. It is also used to make a number of industrial products, which are dependent on the chemical ability of cellulose to unite with acids to form plastic esters. Cellulose nitrate, dissolved in suitable solvents, forms New Skin for the protection of skin abrasions; in another form, cellulose nitrate becomes highly explosive guncotton. Cellulose acetate is a very versatile plastic, which can be molded into many useful articles. It finds its widest application in the manufacture of photographic films. Formerly, photographic films were made of cellulose nitrate, pyroxylin. Fire hazards were so great that pyroxylin film has been abandoned in favor of the slow-burning acetate type.

Cellulose plastics are also used in making various types of lacquers, including automobile lacquers. Cellulose has even invaded the meat-packing industry where artificial plastic sausage casings have replaced, in part, the natural casings.

Straw. This potentially valuable cellulose crop is yet to be exploited. If baled straw could be transported sufficiently cheaply to central processing plants, it could well compete with wood for the manufacture of certain types of paper. It is estimated that there is enough straw available in the United States each year, if collected at proper processing centers, to meet

more than 90 per cent of our annual paper requirements. Annual paper production in the United States is about 21 million tons.

Pilot-plant experiments indicate that straw is suitable for the manufacture of many types of paper products. Since straw contains cellulose and pentosans, it can be used for the manufacture of most of the chemical products now made from wood and similar materials.

Cornstalks. In a general way the above statements regarding industrial uses of straw can also be applied to cornstalks, since they contain celluloses and pentosans. In either case the problem of successful utilization is an economic one. Proponents of the straw-paper process maintain that straw can be processed more cheaply than wood. They maintain that two tons of straw will make 1 ton of paper pulp.

Bagasse. This is the waste fibrous material remaining after the extraction of sucrose from sugar cane. Formerly bagasse was utilized solely as fuel in the sugar mills. Farm chemurgic studies have shown that bagasse can be utilized also for the manufacture of insulating materials, wallboard, and plastic materials. Bagasse can also be used for making the same types of industrial chemicals as are made from wood, straw, and similar crops.

Textile products

Many fiber crops are used industrially for the manufacture of textile fibers. Examples of important fiber crops for textile making are cotton, flax, hemp, jute, and ramie.

Cotton goods. Most of the cotton produced in the United States is made into various types of cotton goods. When cotton threads are treated with alkali and the alkali is removed, the cotton thread takes on a silky sheen and is known as "mercerized cotton."

Linen. This textile fiber is made by "retting" flax straw by microbiological or chemical methods. The flax fibers, which possess great tensile strength, are bleached and spun to form linen cloth. Some linen fiber is produced in the states of Oregon and Washington, but the amount is small compared with our

domestic needs. Most of the flax in the United States is grown in the drier areas of the Middle West and is primarily an oil seed crop.

Hemp and jute. These fiber plants are not grown in commercial amounts in the United States. Hemp and jute fibers are characterized by length and tensile strength. As a result they are used industrially for the manufacture of rope, twine, burlap bags, and special types of carpets and mats.

Ramie. This is an interesting plant which was introduced into the United States from China. The Chinese obtained the plant from India to which it had come, centuries earlier, from Egypt. Ramie has been known as a textile fiber for at least 6000 years. Cloth taken from Egyptian mummies has been identified as ramie cloth. In spite of this, man has been unable to find suitable methods of processing the plant to obtain the excellent fiber in commercial quantities. In Egypt, India, and China the fibers were separated by hand, yielding at best but a few pounds of fiber per man per day.

Until quite recently engineers have been unable to design satisfactory decorticating machines, and chemists have been unable to devise suitable methods of degumming the fibers. Experimental farms and pilot plants have been established in the Florida Everglades. A yield of 60,000 pounds of green ramie plants from 1 acre will yield 1800 pounds of decorticated gummy fiber and about 58,200 pounds of leafy and stemmy residues. Chemists are searching for methods of utilizing these residues. The only outlet at present is as a green, dried meal for livestock feeding. It is also an excellent source of commercial chlorophyll.

Degummed ramie fibers are long, white, and silky. When dry they are the strongest of all natural fibers, and when wet they are stronger than any known textile fiber, except glass fibers. In fact the tensile strength of wet ramie fibers is nearly four times that of wet cotton or wet nylon fibers. For these reasons this fiber offers considerable promise as a material for the fabrication of awnings, belting, canvas, cordage and twine, fire hose, hammocks, sailcloth, upholstery webbing, marine cordage, fishlines, fish nets, and similar materials. Ramie fiber is also suitable for curtains, draperies, dress goods, and suitings.

Industrial chemicals

Most of the chemicals produced from woody or fibrous plants are derived from the hydrolytic products of the various polysaccharides. Cellulose yields glucose, a primary raw material, and the pentosans yield pentose sugars (xylose).

Wood distillation. The principal products of wood distillation are acetic acid, wood alcohol (methanol), wood tar, and charcoal. Owing to inefficient methods of wood distillation and to the synthetic production of acetic acid and methanol, the wood distillation industry has had difficulty in surviving. It is not a large industry, and its products are not in great demand.

Wood sugar. When wood waste is treated with sulfuric acid and water and heated under pressure, the cellulose is hydrolyzed to glucose. This dilute sugar solution can be condensed to molasses, containing 85 per cent glucose, which is sold as an energy supplement for mixing with livestock feeds of low energy content. It can be crystallized to form crude wood sugar. The dilute solution can be fermented with yeast for the production of industrial alcohol (ethanol), or it can be used as a nutrient medium for the production of high protein (*Torula*) yeast which is known as "food yeast." This yeast is a good source of protein and vitamins for the feeding of domestic livestock. During World War II this type of yeast, grown on wood sugar, served as an important protein food for German soldiers and civilians. A palatable "ersatz" sausage made with cereals and yeast was a regular issue item in the German army. Alcohol and food yeast can also be made from the sulfite liquor which results from the manufacture of paper pulp. In fact any chemical that can be made from starch or glucose can also be made from wood sugar.

Furfuraldehyde. This important industrial chemical can be made from many types of woody plants, such as wood residues, corncobs, and oat, rice, and cottonseed hulls. To date most commercial furfural is derived from oat hulls by methods devised by the Miner Laboratories and the Quaker Oats Company.

Furfuraldehyde is derived from the pentose sugars present in the vegetable pentosans. Vegetable wastes, such as corncobs or

oat hulls, are digested with sulfuric acid, and the furfuraldehyde is obtained by distillation. This chemical has come into wide use in industry, although it was almost a chemical curiosity for a number of years. As soon as it was available in quantity, experimental work found many applications for this versatile chemical. It is employed as a preservative by morticians and as a degumming solvent by petroleum chemists. In fact, the petroleum industry is furfural's largest customer. Furfural can be polymerized with phenol to form a synthetic plastic which has found almost unlimited uses in industry. Furfural is also a starting chemical for the synthesis of other chemical compounds.

E. I. du Pont de Nemours and Company has prepared more than 100 derivatives, using furfuraldehyde as a starting material. A large group of nitrofurans derivatives have been prepared by the Eaton Laboratories at Norwich, New York. One compound, nitrofurazone, is said to have marked bacteriostatic properties. Du Pont chemists have utilized furfural as the starting material for the synthesis of the essential amino acid, lysine.

Adiponitrile, an essential intermediate in the manufacture of nylon, is made from furfuraldehyde. Adiponitrile is hydrogenated to form hexamethylenediamine and combined with adipic acid to form nylon salt, which yields the commercial nylon fiber.

INDUSTRIAL USES OF PROTEIN

The nutritional importance of proteins will be discussed in a subsequent chapter. Our present discussion will deal with non-food industrial uses of these important nitrogenous compounds. Proteins from different vegetable sources are so similar in chemical and physical properties that they can be used interchangeably for many industrial purposes. The most important industrial use of proteins is for the production of plastics, adhesives, coatings for paper products, as binders for inert materials in molded plastic articles, in bonding plywood veneers, and as artificial textile fibers.

Sources of industrial proteins include such vegetable products as soybean, cottonseed, and peanut residues remaining after the respective vegetable oils have been extracted. Casein from skim

milk is another important industrial protein used for the manufacture of adhesives, plastic coatings, molded products, and as a constituent of casein paints.

A fiber has been made from casein by extruding the plasticized product. This casein fiber can be woven into a fabric known as "casein wool." Other proteins can also be made to form similar types of textile fibers. Unfortunately the protein fibers have low tensile strength and tend to disintegrate when wet. These facts, coupled with the fact that proteins are relatively expensive, leads to the unavoidable conclusion that plastic protein fabrics will not be able to compete with the more durable fabrics of commerce. Wool is a natural protein fiber which owes its durable qualities to a high content of a tough insoluble protein, *keratin*.

Protein hydrolyzates are being sold in increasing amounts for the treatment of protein deficiencies in medical, surgical, and obstetrical cases. These preparations have found wide use in those cases where high protein diets are required. When patients are unable to take food by mouth, protein hydrolyzates may be administered by parenteral injections, thus furnishing a continuous supply of amino acids in predigested form.

INDUSTRIAL USES OF NATURAL CHEMICAL PRODUCTS

There are many naturally occurring types of chemical products produced by plants which can be removed with little or no chemical change by means of extraction with chemical solvents, by steam distillation, or by mechanical means. These chemical substances can be purified and used directly, they may be utilized as starting materials for the synthesis of other chemicals, or they may be mixed with other chemical substances for special purposes. Following are a few examples of naturally occurring compounds used for industrial purposes.

Turpentine, rosin, and pine oil

These products are known as *naval stores* because, in the days of sailing vessels, pitch, tar, and turpentine were used to paint

and calk wooden ships to make them watertight. Southern long-leaf pines, grown from North Carolina to Florida and Texas, are the principal sources of naval stores in the United States.

The living trees are scarified from time to time, and the exuding resinous liquid or gum is collected in small cups which are emptied at frequent intervals. Yields of gum can be increased by treating the scarified areas with sulfuric acid. The crude exudate is steam distilled and the turpentine is removed, leaving rosin as a non-volatile residue.

Old pine stumps are also utilized for this purpose. They are transported to factories where they are shredded and destructively distilled in retorts similar to those used for the destructive distillation of hard woods. Products obtained by this method are light oils, turpentine, dipentene (dipentene), pine oil, tar oil, and pine tar. Charcoal remains as a residue in the retort. Steam distillation and solvent extraction methods are also used to remove chemical materials from shredded stump wood.

Pine oil is used in pharmaceuticals, floor waxes, furniture polishes, soaps, and similar products. Turpentine is employed as a solvent, and the various resins, made from rosin, are used in laundry soaps and for other purposes. These resins may be polymerized and utilized as plastic adhesives, binders, and coatings. They are also used in paints, varnishes, and synthetic rubber tires.

Tannins

These chemical substances are used for the tanning of hides in the leather industry. Originally about 60 per cent of our domestic tannins came from the wood of the chestnut tree, and the remainder was furnished by oak and hemlock bark and sumac leaves. Owing to the almost complete extinction of the chestnut tree by blight our present supply of tannins must come, largely, from dead chestnut wood. This supply will soon be exhausted. Consequently there is a serious shortage of domestic tanning materials, and the American leather industry is forced to obtain most of its needs from foreign sources.

Potential sources of tannins, now being investigated by Federal research scientists, are various varieties of sumac, scrub oak

barks, red mangrove bark, and barks from hemlocks in eastern, western, and Great Lakes states.

Essential oils

Mint oils. A number of essential or volatile oils are produced in the United States. Peppermint and spearmint production is centered in the states of Michigan and Indiana, although Oregon and Washington are beginning to challenge these states for first place in essential oil production.

The yield of mint leaves is from 2 to 3 tons per acre, which amounts to about 30 pounds of essential oil per acre. Initial steam distillation is usually conducted on the farms, and the crude oils are refined in industrial laboratories. The annual production of peppermint oil is in excess of one million pounds. Spearmint oil production is about one-fourth of peppermint oil production. These oils are used as flavoring materials in candy and chewing gum, and, to a less extent, for condimental purposes. Mint oils are the source of *menthol*, a white crystalline compound used in pharmaceutical and cosmetic preparations and as a flavoring material in tobacco.

Citrus oils. Essential oils are obtained from citrus fruits by cold pressing the pulp-free peel. If gums, waxes, and terpenes are removed, the pure, concentrated oils can be used as food flavors. *Citric acid*, also a by-product of the citrus industry, is usually added with the oils in order to simulate the flavor of the original fruit.

Sassafras oil. This essential oil is distilled from the root bark of sassafras plants. It contains an alcohol, *saffrol*, which is the principal flavoring agent in root beer and in some types of candy.

Oil of wintergreen and sweet birch oil. Both of these essential oils owe their flavor and properties to *methyl salicylate*, which constitutes about 90 per cent of these oils. Oil of wintergreen is distilled from teaberry leaves or from leaves of partridge berry plants; oil of birch is distilled from birch bark. These oils are also used as flavoring materials.

Miscellaneous essential oils. Other essential oils derived from domestic plants are oil of dill (seeds), sage, thyme, and marjoram.

Some are used as food flavors, and others are used as tobacco flavors.

Pigments

Crystalline *carotene* is an important medical and pharmaceutical chemical extracted from highly pigmented carrots grown commercially for this purpose. Commercial carotene consists largely of β -carotene with small amounts of α - and γ -carotene. These pigments can also be extracted from many types of leafy plants and from sweet potatoes. In a later chapter we will learn that carotene is the parent substance or precursor of vitamin A. The carotenoid pigments are used in pharmaceutical preparations and as vitamin A supplements for proprietary foods.

Chlorophyll is also extracted from green leafy plants and sold to chemical and pharmaceutical houses.

Insecticides

Rotenone. This compound has been discussed in a previous chapter. The rotenoid poisons are found in a number of tropical plants. Rotenone is extracted from derris and *Lonchocarpus* roots, which contain from 8 to 13 per cent rotenone. The poison is marketed as a dusting powder or as a liquid spray for insect control.

Pyrethrum. This plant has been mentioned in a previous paragraph. From a chemurgic standpoint it is of interest because pyrethrum production has been attempted in the United States. The flowers must be harvested by hand, which is an economic handicap. The extracted pyrethrins are marketed as insecticide dusts, insect powders, and sprays. It is possible that synthetic pyrethrins may eventually replace the natural products.

Rutin

This compound can be extracted from a number of plants, including tobacco and buckwheat leaves. The latter is the preferred commercial source because of higher yields per weight of dried leaves. The drug, rutin, has shown considerable promise for the treatment of capillary fragility and hemorrhagic diseases.

Dr. J. F. Couch, an authority on rutin, states that at least fifteen drug companies are manufacturing rutin from buckwheat, and it is estimated that the demand for the drug will call for more than 50,000 acres of buckwheat annually. This drug was isolated in the laboratories of the Federal Eastern Regional Research Laboratory. Cooperative experiments are being conducted on buckwheat varieties at the Pennsylvania State College, to study soil, fertilizer, climatic, and other factors that might affect rutin synthesis by the buckwheat plant.

Other drugs

Cascara, an ingredient of many proprietary laxative preparations, is extracted from cascara bark obtained from the cascara buckthorn (bearwood), grown on the Pacific coast. *Wormwood*, used in liniments and as an ingredient of absinthe, is an oil obtained by steam distillation of wormwood, a perennial grown in Indiana and Michigan.

COMMERCIAL UTILIZATION OF PACKING-PLANT RESIDUES

In our discussion of cellulose crops it was pointed out that waste residues such as oat hulls, corncobs, straw, and cornstalks can be utilized for commercial purposes. The same is true for nutshells from peanuts, pecans, and almonds. The concluding section of this chapter will deal with wastes from food-processing plants.

Vegetable wastes

Cannery waste disposal is a vital problem facing the food-processing industry. These wastes must be disposed of in such a way that they will not become a public nuisance or bring about stream pollution. Naturally, the food industry would like to find ways and means of utilizing these wastes for some useful and profitable purpose. To date results have not been particularly satisfactory, and much research remains to be done on this problem.

Dr. J. J. Willaman of the Eastern Regional Research Laboratory has shown that leafy wastes from vegetable-packing plants can be dried and ground to form a nutritious meal for the feeding of poultry and livestock. In Florida, waste lettuce, celery, citrus peel and pulp, and tomato skins and seeds are dried and fed to livestock. The tonnage of pea vines and pods and leaf and stem wastes from vegetable-processing plants is enormous. It is conceivable that dried meals made from these residues can become important sources of protein, carotene, and riboflavin for livestock.

Miscellaneous residues

The citrus-packing industry has been relatively efficient in utilizing citrus peel, rag, and seeds for the manufacture of stock feeds, essential oils, and pectin. Residues from apple canneries, such as skins and cores, have also been utilized for the manufacture of commercial pectin and for the production of jellies, apple butter, and vinegar. The dried apple pomace can be fed to livestock.

Argol, a residue which settles out during the manufacture of grape juice and wine, is sold as a source of tartaric acid. To date, however, no extensive industrial use has been found for the skins, seeds, pulp, and stems left in the grape press cake.

Fish-processing plants have been quite efficient in utilizing fish wastes. Scraps, wastes, and stick liquor from fish are dried and used as valuable sources of protein and fat-soluble vitamins for livestock. Riboflavin concentrates, for livestock feeding, are also made from fish wastes.

Probably no American industry is more efficient in completely utilizing its products and by-products than the meat-packing industry. Every tissue is utilized for some purpose. These include hides, horns, bones, hoofs, hair, ductless glands, blood, and viscera. Tissues not used for foods or pharmaceuticals are dried and sold as tankage for livestock feeding or as fertilizer.

For more detailed discussions of the chemistry and technology of farm chemurgic processes, the reader is referred to the following references.

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Part 3 ·
The Animal

15 • Foods and Feeding Stuffs

The animal body must receive in the food all nutrients necessary for the construction and repair of metabolizing tissues and, in addition, sufficient oxidizable substances to furnish the various forms of energy required by the body. Although the animal body is often compared with an internal-combustion engine or similar machine, there is at least one essential difference. When fuel is burned in an internal-combustion engine, heat and mechanical energy are produced from the chemical energy of the fuel. When food is eaten by the animal, the same phenomenon occurs but with the essential difference that the food also contributes to the manufacture of new tissues and to the repair of old ones, whereas fuel cannot contribute to new cylinders and pistons in the engine. Therefore the engine wears out and deteriorates whereas the living machine is continuously being remade with new materials.

A complete food might be defined as one that will contribute to every need of the body when this food is the sole article of diet for an appreciable period of time. It is impossible to find a single foodstuff that will meet the exacting requirements of a healthy body. Perhaps milk most nearly approaches this ideal, but we shall find later that milk may be lacking in a number of essential factors.

Most of the foodstuffs that we appreciate and enjoy are deficient in many respects. It is for this reason that man has found by experience that dietary diversity encourages health and growth, whereas the reverse is true if one-sided diets are ingested for appreciable periods of time.

In subsequent chapters we shall learn why diversified diets

are nutritionally superior and why two or more qualitatively inadequate foods can supplement each other in forming a nutritionally improved mixture. As our studies progress, we will discuss the biological value of the various food constituents, and we will also consider the fate of these substances during metabolism.

In this chapter we shall devote our attention primarily to the sources and chemical composition of some of our more important foods and feeds.

DEFINITION OF TERMS

When feeding stuffs are purchased on the market, pure food laws require that such feeds be sold on the basis of chemical composition. As a result it is customary to make the following chemical analyses on all feeding stuffs:

1. Moisture.
2. Crude protein.
3. Crude fat.
4. Crude fiber.
5. Ash.
6. Nitrogen-free extract.

Moisture. It is necessary to determine the moisture content of foods and feeding materials in order that we may have more accurate methods of comparing one feed with another. As a rule feeds are usually fed and compared on what is known as a *moisture-free* basis in order that comparisons may be made on the basis of dry matter or total solids. Certain feeds deteriorate rapidly if the moisture content exceeds certain limits, which vary for different feeds. For these reasons knowledge of the water content is highly desirable. Furthermore a feed buyer cannot afford to buy expensive feeds and pay feed prices for water. As a result the buyers of such commodities often prescribe the maximum water the materials may contain.

Crude protein. Since all proteins contain about the same amount of nitrogen (16 per cent), it has become the practice of laboratories to determine the total nitrogen present in the feed and multiply this amount by the factor 6.25. This general

factor is used for most proteins, although there are exceptions to this rule. For example, the factor 5.7 is used for the calculation of crude protein in wheat and its products, since the proportion of nitrogen in wheat proteins is greater (about 17.5 per cent) than in most proteins. The nitrogen is determined by the Kjeldahl method, oxidizing the feed completely by boiling in sulfuric acid. This liberates the nitrogen in the form of ammonia, which combines with the acid, forming ammonium sulfate. By the addition of a strong alkali (NaOH) the ammonia is liberated and is distilled into an acid of known strength, from which the amount of ammonia present may be calculated, after the titration of the excess acid. By further calculation the amount of nitrogen is determined, which, when multiplied by the factor 6.25, gives the amount of crude protein present. The term "crude" is used advisedly, since this method determines the total organic nitrogen, regardless of source. There are, of course, small amounts of nitrogenous compounds present in feeds which are not proteins. For this reason the term "crude protein" is applied.

Crude fat or ether extract. When dry feeds are extracted with anhydrous ethyl ether, all ether-soluble materials are extracted. This extract, consisting mainly of glycerides of the fatty acids, is weighed and the percentage computed. Since, however, the ether extract contains appreciable amounts of sterols, pigments, and other ether-soluble materials, the residue has been given the name "crude fat" or "ether extract."

Crude fiber. When a feed is boiled, consecutively, with sulfuric acid (1.25 per cent) and sodium hydroxide (1.25 per cent), the proteins, fats, and most carbohydrates are hydrolyzed, leaving, after filtration, the more resistant woody material (fiber). This is dried, weighed, and burned. The loss in weight, on burning, is assumed to be the indigestible portion of the feed and is known as "crude fiber." Although it is an index of the amount of indigestible matter in the feed, it is only approximately true, for the reason that ruminating animals and horses can utilize appreciable amounts of crude fiber. Nevertheless it is the least valuable portion of the feed and for this reason should be kept at a minimum in those feeds sold on the market as concentrates.

Ash. A weighed portion of a feed is ignited in a muffle furnace or over a gas flame until the carbonaceous matter is completely oxidized and the mineral salts remain in the crucible or dish as a white or grayish white ash. This ash does not truly represent the mineral salts in the feed, since many of the salts are changed during the heating process. Organic substances containing such elements as sulfur and iodine are destroyed, and varying amounts of these elements may be lost by volatilization. If the temperature of heating is sufficiently high, it is possible that other elements such as sodium and potassium may be lost also. Usually, however, the temperature is controlled to prevent the volatilization of these bases. The ash of feeds contains the basic elements combined as oxides, carbonates, and phosphates, although these bases may not have existed in these forms in the feed. Therefore, the ash determination is a rough index of the true mineral content of the feed.

Nitrogen-free extract. When the chemist has determined all the above-mentioned constituents and has calculated them in terms of percentage of the moisture-free feed, he adds these percentages and subtracts the total from 100. The difference is the so-called "nitrogen-free extract," which is composed almost entirely of carbohydrates, although small amounts of certain organic acids and similar materials are included. For practical purposes nitrogen-free extract may be considered synonymous with carbohydrates.

The Federal Food and Drug Act of 1906 was enacted to protect the American public and to regulate the manufacture and sale of foods, feeds, and drugs sold in interstate commerce. Standards for each type of commodity were established by law. Today most of the states in the United States also have enacted food laws controlling the purity and quality of foods and feeds manufactured or sold within their respective borders.

So far as laws relative to commercial feeds are concerned, all of them express feed composition in terms of moisture, crude protein, crude fat (ether extract), crude fiber, ash, and nitrogen-free extract, as described above. In order that the feed buyer may be protected, most feeding-stuff laws require that the manufacturer submit a guarantee regarding the chemical com-

position of the feed. If the feed inspection laboratories find that a product does not conform to the guaranteed analysis, the manufacturer and dealer are subject to legal action.

Many animal feeds differ in chemical composition from those foods commonly used for human consumption. This is due to the fact that domestic animals are capable of relishing and utilizing many products that the human digestive tract cannot tolerate.

STOCK FEEDS OF PLANT ORIGIN

For convenience of classification we shall discuss feeding stuffs from the standpoint of source of material. Considering first the feeds that are obtained from plant sources, we have:

1. Seeds or cereals (concentrates).
2. Stem and leaf crops (fodders).
3. Vegetables (succulent feeds).

Seeds or cereals. In general it may be said that seeds used for animal feeding can be divided roughly into three classes: (1) cereal grains, (2) oily seeds, and (3) leguminous seeds.

Cereal grains. From the standpoint of tonnage this group is probably the most important; it consists of such cereals as wheat, corn, barley, oats, and similar seeds.

PER CENT COMPOSITION OF SOME TYPICAL CEREAL GRAINS *

Feeding Stuff	Dry Matter	Ash	Proteins	Carbohydrates		Fat
				Fiber	N-Free Extract	
Corn, dent No. 1	87.0	1.2	8.8	2.1	70.9	4.0
Oats, whole	90.2	4.0	12.0	11.0	58.6	4.6
Wheat	89.5	1.9	13.2	2.6	69.9	1.9

* See the Appendix for tables showing composition of representative human foods and livestock feeds.

In addition, factory by-products such as brans, middlings, gluten feeds, and distiller's grains are sold on the market as products to be used as ration ingredients.

PER CENT COMPOSITION OF SOME CEREAL BY-PRODUCTS

Feeding Stuff	Dry Matter	Protein	Fat	Carbohydrates		Ash
				Fiber	N-Free Extract	
Brewer's grains	92.9	27.6	6.5	14.3	40.9	3.6
Corn gluten feed	90.9	25.5	2.7	7.6	48.8	6.3
Distiller's corn grains	92.9	28.3	8.8	11.4	41.9	2.5
Distiller's corn solubles	93.0	26.7	7.9	2.6	48.4	7.4
Winter wheat bran	89.9	15.5	4.2	8.9	55.1	6.2
Wheat middlings	89.7	18.0	4.7	7.4	55.1	4.5

In a general way the cereal grains may be said to be characterized by a relatively high content of carbohydrates (nitrogen-free extract) which is largely starch. Although the cereal grains are not poor in protein, they cannot be considered protein-rich. The protein of cereals is not considered to be equal, in biological value, to proteins obtained from legumes or from animal products. As a rule the cereals are not rich in fiber, fat, or mineral salts. They are lacking particularly in calcium.

Oil-containing seeds and leguminous seeds. Cottonseed, linseed and soybean are three important oil-containing seeds used in livestock feeding. As a rule they are seldom fed as ground whole seeds because of their high content of oil. The oils are removed by pressure or extraction, and the by-products are sold in the form of cake or meal for feeding purposes. These meals are characterized by high protein content and are important as concentrates in raising the protein content of grain rations. Soybean meal or cake has been used in increasing amounts during the past few years. Soybean meal from the corn belt may contain as much as 45 per cent protein; southern soybean meal may contain 48 per cent or more. Since soybean meal is

low in fiber content, it is better utilized than cottonseed or linseed meals. Soybean protein also possesses superior biological qualities, owing to a better distribution of essential amino acids. Cottonseed meal contains a toxic substance called gossypol, which varies in amount, depending upon climatic and soil conditions. Cattle are able to ingest large amounts of cottonseed meal without showing ill effects, but other types of livestock are more susceptible. Fortunately much of the gossypol present in the cottonseed is inactivated during the heating process employed during the manufacture of cottonseed oil. It is now thought that previously reported cases of cottonseed poisoning in cattle were caused by dietary deficiencies, largely vitamin A deficiency, rather than by gossypol toxicity. Peanut and sunflower meals are also used to a limited extent as protein concentrates.

PER CENT COMPOSITION OF SOME TYPICAL OIL MEALS

Feeding Stuff	Dry Matter	Ash	Proteins	Carbohydrates		Fat
				Fiber	N-Free Extract	
Cottonseed meal (Texas)	92.5	5.8	42.7	10.6	27.0	6.4
Linseed meal	91.0	5.6	35.4	8.2	36.0	5.8
Peanut meal	93.0	5.2	43.5	13.3	23.4	7.6
Soybean meal	91.2	6.1	44.6	5.8	29.4	5.3

Stem and leaf crops or fodder crops. In the fresh green state these foods contain relatively large amounts of water and, consequently, small amounts of total solids or dry matter. After drying they are characterized by a relatively high amount of crude fiber and ash, but the amount of carbohydrates is relatively low. The protein will vary, depending on the type of plant under consideration. Dry alfalfa hay or other legume hays, for example, are quite rich in protein, whereas the grass hays are relatively poor in this regard.

Silage, most commonly made from corn, is another member of this group. It is a most important feed and varies in composition, depending on the maturity of the corn and on the presence of other crops, such as clover or soybeans, which are used with corn in silage manufacture.

PER CENT COMPOSITION OF SOME TYPICAL FODDER CROPS

Feeding Stuff	Dry Matter	Ash	Pro-tein	Carbohydrates		Fat
				Fiber	N-Free Extract	
Alfalfa hay	90.4	8.3	14.7	29.0	36.4	2.0
Clover hay	89.0	7.9	12.0	27.1	39.8	2.2
Corn stover	90.6	5.8	5.9	30.8	46.5	1.6
Timothy hay	88.7	5.0	6.2	30.1	45.0	2.4
Wheat straw	90.1	8.2	3.8	35.7	40.9	1.5

Vegetables used as feeds. In many parts of this country and of Europe root crops are raised as feed for livestock. Most of these, such as mangels, sugar beets, rutabagas, and turnips, are root crops, but other types of vegetables, such as pumpkins, squash, cabbages, and kale, are raised for this purpose. These feeds are valued for their succulent qualities. They are fed mainly for their vitamins, mineral salts, and carbohydrates, although they do contribute in some degree to the protein intake.

STOCK FEEDS OF ANIMAL ORIGIN

Feeds of this type can be classed in two large groups: (1) those consisting of milk or milk by-products and (2) those consisting of packing-house by-products.

Prior to World War II milk products and packing-house by-products were available at prices that permitted some feed manufacturers and livestock feeders to include appreciable amounts of these valuable concentrates in proprietary and in home-mixed

feeds. During the war it became necessary to conserve skim milk for the feeding of the armed services, for lend-lease, and for feeding civilian populations over the world. As a result there has been a shortage of these materials for livestock feeding. At present (1950) prices most milk products are too expensive for livestock feeding, with the possible exception of certain types of poultry feeding. Condensed and dried buttermilk, whey, and dried skim milk (of a quality not suitable for human consumption) are still used extensively by poultry feeders.

Milk and milk by-products. When it is economically feasible, whole milk, skim milk, and buttermilk are important feeds for growing livestock. They are obtainable also in evaporated and powdered forms. Whey, as a by-product of cheese making, is also a feeding material, but its use has been confined mainly to hog feeding and poultry feeding.

Composition of milk. It is necessary that we consider some of the outstanding characteristics of milk, since it is the most nearly complete food known to science. Milk is the normal secretion of the mammary gland; although there are many kinds of milk, all references, unless otherwise indicated, will be to cow's milk.

PER CENT COMPOSITION OF MILK FROM DIFFERENT SPECIES

	Water	Casein	Albumin	Fat	Lactose	Ash
Human	87.41	0.91	1.23	3.76	6.29	0.31
Cow	87.27	2.95	0.52	3.66	4.91	0.69
Goat	84.14	3.04	0.99	6.00	5.02	0.81
Sheep	81.90	4.57	1.26	6.52	4.82	0.93
Camel	87.04	3.49	0.40	2.76	5.57	0.74
Buffalo	82.14	4.29	0.49	7.44	4.81	0.83
Horse	90.68	1.27	0.75	1.17	5.77	0.36
Ass	89.88	0.73	1.31	1.50	6.09	0.49
Reindeer	68.20	8.40	2.00	17.10	2.08	1.50

Cow's milk varies in composition, particularly in fat, depending on the breed of cow from which the milk is obtained. Other

factors, such as inheritance, individuality, time of milking, and period of lactation, also influence the composition of milk.

EFFECT OF BREED ON MILK COMPOSITION
(Expressed in Per Cent)

Breed	Water	Protein	Fat	Lactose	Ash	Total Solids
Jersey	85.27	3.80	5.14	5.04	0.75	14.75
Guernsey	85.45	3.84	4.98	4.98	0.75	14.55
Ayrshire	87.10	3.34	3.85	5.02	0.69	12.90
Shorthorn	87.43	3.32	3.63	4.89	0.73	12.57
Holstein	88.01	3.15	3.45	4.65	0.68	11.93

Although it is possible to affect the amount of milk produced by underfeeding and overfeeding, it is not possible to produce marked changes in the fat, protein, or sugar content of milk by changes in feeding practice. It is possible, however, within narrow limits, to change the nature of some of the ingredients. For example, the feeding of certain feeds tends to produce butterfat which varies in its melting point, but efforts to increase the percentage of butterfat by feeding methods have not met with commercial success.

Milk proteins. Examination of the previous table on milk composition shows that the total solids of cow's milk consist largely of butterfat, proteins, lactose, and inorganic salts. Milk also contains carotene, vitamins A and D, riboflavin, thiamine, and ascorbic acid.

From a nutritional standpoint the proteins of milk are of great importance since they contribute essential amino acids necessary for normal health and growth. The proteins of milk are casein, lactalbumin, and lactoglobulin. Casein is present in the largest amount and represents that portion of the milk which precipitates when milk becomes sour. This precipitate is called the "curd" and is marketed as cottage cheese.

Lactalbumin is a water-soluble protein which is coagulable by heat, and, although it rarely occurs in excess of 0.5 per cent

of the milk, it constitutes about one-sixth of the total milk protein. Lactoglobulin is a water-soluble protein which exists in normal milk in very small amounts.

Milk fat. Butterfat is a highly palatable mixture of glycerides of fatty acids. The unique flavor of butterfat is attributed to the presence of diacetyl (dimethylglyoxal) which is formed during the ripening of the cream. Diacetyl is sold as a commercial chemical, and it is used as a flavoring agent to enhance the flavor of butters and margarines. The unique physical properties of butterfat result from the types of fatty acids combined as glycerides. Butterfat is characterized by the presence of a relatively large amount of short-chain fatty acids such as butyric, caproic, caprylic, and capric acids.

From the viewpoint of nutrition it is unfortunate that the market value of milk is based solely on its butterfat content. From an energy standpoint butterfat has no advantage over several other edible fats and oils. Butterfat does make important contributions to the vitamin A content of the diet, but the carotene and the vitamin A content of butter is subject to wide seasonal variations. Vitamin A can be obtained just as economically from other natural foods, particularly leafy vegetables, eggs, and whole milk. In the opinion of the authors of this book milk should be bought and sold on the basis of the most valuable nutrients, i.e., non-fat milk solids. It is the non-fat solids which contain the essential proteins and indispensable mineral salts and vitamins. It is for these reasons that the nation's supply of dried whole and skim milk was requisitioned by the Federal Government during World War II in order to ensure better nutrition for the armed services and for civilian populations here and abroad.

Milk sugar. The chemistry of lactose has been discussed in a previous chapter. This sugar is not hydrolyzed by adults so efficiently as other sugars. Infants and young animals seem to be able to hydrolyze lactose to glucose and galactose quite efficiently. Lactose forms lactic acid in the intestine, thereby favoring the development of desirable types of acid-forming organisms which discourage the development of putrefactive organisms. One of the important functions of lactic acid is to promote the utilization of calcium and phosphorus by increasing

the intestinal absorption of these important mineral elements.

Mineral salts. One of the outstanding characteristics of milk is the fact that it contains mineral salts in approximately the same proportions as they exist in the body of a newborn animal. Milk ash contains potassium, calcium, sodium, magnesium, iron, phosphorus, chlorine, and sulfur, as well as traces of copper, zinc, aluminum, manganese, and iodine. Citric acid and carbonic acid are important acids, combining with bases to form salts. Other salts thought to exist in milk are chlorides and phosphates.

Other constituents. It would appear that the chemical constituents of milk are in complex equilibrium, and much is yet to be learned regarding the forms and combinations in which they exist. It is thought by many authorities that potassium, sodium, chlorine, and lactose are entirely in solution, whereas the casein and the fat are present entirely as emulsoids and colloidal sols. Some writers are of the opinion that calcium exists in milk as calcium caseinate. Others are of the belief that calcium caseinate and calcium phosphate may combine in chemical or physical combinations. When milk is heated, very small amounts of the salts, including calcium and phosphorus, may be lost in a sludge or residue that settles out. There is little evidence that the soluble calcium and phosphorus salts are changed to insoluble salts by heat. It is possible that the coagulation of albumin by heat may trap salts mechanically and carry them out of solution. Lactalbumin, calcium, magnesium, and phosphorus are found partly in true solution and partly in suspension. Market milk always contains dissolved gases such as carbon dioxide, nitrogen, and oxygen, which have been introduced subsequent to milking. These may be introduced by aeration or by the action of bacteria, although milk drawn from the udder contains about 10 per cent (by volume) of carbon dioxide which usually diminishes during subsequent handling and pasteurization.

Lecithin and cholesterol are also normal milk constituents. The former ranges from 0.03 to 0.05 per cent, whereas the latter fluctuates with the fat and may vary from 105 to 176 parts per million of raw milk. Other constituents are enzymes, of which protease, lipase, phosphatase, catalase, peroxidase, dehydrogenase, and a lactose-fermenting enzyme are the most important.

Milk products. The following table shows the approximate chemical composition of some of the more important products made from milk.

APPROXIMATE COMPOSITION OF SOME IMPORTANT MILK PRODUCTS
(Expressed in Per Cent)

Product	Water	Pro- tein	Fat	Lac- tose	Ash	Su- crose	Salt	Lac- tic acid	Curd
Powdered whole milk	2.00	26.91	28.65	36.50	5.94
Evaporated whole milk	73.63	6.71	8.22	10.13	1.55
Sweetened condensed milk	27.03	7.85	8.99	12.65	1.76	41.65
Whole milk	87.30	3.55	3.62	4.82	0.71
Light cream	72.46	2.95	20.00	4.00	0.59
Heavy cream	63.41	2.58	30.00	3.50	0.52
Whipping cream	54.35	2.21	40.00	3.00	0.44
Skim milk	90.36	3.72	0.15	4.98	0.80
Evaporated skim milk	71.05	11.16	0.45	14.94	2.40
Powdered skim milk	3.89	35.42	1.74	48.74	8.08
Butter	13.90	82.41	2.51	1.18
Buttermilk	91.61	3.30	0.50	3.40	0.65	0.50
Condensed buttermilk	60-64	12-15	1-2	16-20	2.5-3.5	2-3
Dried buttermilk	1.93	38.74	5.87	39.91	7.68	5.87
Cheddar cheese	37.33	23.39	33.41	7.02
Domestic Swiss cheese	30-34	26-30	30-34	3-5	1-1.4

Packing house by-products. By-products of the meat-packing industry have been used extensively as protein supplements in rations for domestic livestock, and, in spite of the present (1950) high cost of these materials, they are still considered essential for successful livestock feeding. Concentrates of this type which are most commonly used include tankage, meat meal, meat scraps, blood meal, and pork cracklings.

Fish meal and ground dried fish. These products are made from waste materials from fish-packing plants or from types of fish that are not acceptable as human food. Fish products are valuable as protein supplements in many types of livestock rations. Fish products are valuable also as sources of easily available calcium and phosphorus because they contain constant and appreciable amounts of ground fish bone. Marine fish are also rich in iodine.

Packing house by-products and fish meals are valued primarily for the biological value of the animal protein they contain. Animal proteins are biologically superior to plant proteins because they contain better combinations of the essential amino acids required by animals for normal growth and reproduction.

PER CENT COMPOSITION OF SOME TYPICAL PACKING-HOUSE
BY-PRODUCTS

Feeding Stuff	Dry Matter	Protein	Fat	Carbohydrates		Ash
				Fiber	N-Free Extract	
Blood meal	92.2	84.7	1.0	1.1	0.7	4.7
Fish meal	92.9	63.9	6.8	0.6	4.0	17.6
Meat scraps	93.9	55.8	9.3	2.1	1.3	25.4
No. 1 tankage	93.1	60.6	8.5	2.0	1.8	20.2

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16 · Digestion of Foods

In the previous chapter we considered some of the characteristics of foods and feeding stuffs with regard to chemical composition. We observed that some foods are fed mainly for the protein they contain; others are valuable for their content of energy-producing nutrients (fats and carbohydrates); other foods are characterized by containing mineral elements in abundance; and others contain relatively large amounts of cellulose, which is digested less readily than the other nutrients. We shall now direct our attention to the fate of the various nutrients as they undergo digestion in the alimentary tract. *Digestion may be defined as the process whereby the inorganic and organic nutrients are made soluble and diffusible in order that the nutrients may be utilized for the repair and building of tissue and for the transformation of energy.*

In Chapter 1 we referred to the willow-tree experiment of van Helmont, the versatile Belgian physician, who is well known for his early attempt to find the "principle of vegetation." Van Helmont is best known, however, for his work in medicine, and it was he who first suggested that the processes involved in digestion might be analogous to those of fermentation. Following van Helmont's explanation many theories were advanced to explain the various mechanisms involved in the digestive process. One theory, advanced by Dr. Archibald Pitcairn in the seventeenth century, explained the digestive process on a purely mechanistic basis. Dr. Pitcairn believed that the process was largely mechanical in character, the enormous power of the stomach walls being sufficient to grind foods to particles of infinitesimal fineness, in which form they were utilized by the body.

However, this theory was overthrown when William Stevens,

using a man who was able to regurgitate his food at will, fed to the experimental subject food which had been packed in hollow metal balls perforated with many holes. When the balls were regurgitated, it was found that the food had disappeared and the balls were empty. Stevens and Spallanzani were able to show, independently, that gastric juice after removal from the body was capable of digesting food.

In the early part of the nineteenth century (1822) the entire matter was settled in a conclusive manner by the classical experiments of an American army surgeon, Dr. William Beaumont. The doctor was called to treat a Canadian soldier, Alexis St. Martin, who had been wounded in the left side by the accidental discharge of a musket. On healing, the wall of the stomach adhered to the abdominal wall in such a manner that a permanent opening, known as a *gastric fistula*, remained. It was possible for Dr. Beaumont to introduce food and extract gastric juice from the opening. Recognizing this unusual opportunity he persuaded the authorities to allow him to use St. Martin as an experimental subject.

As a result Dr. Beaumont established the true facts regarding gastric digestion, and most of his observations have been confirmed by subsequent research. He described the appearance of the stomach walls during gastric secretion; he described the gastric juice and noted its acid properties; he studied the excitation of the stomach by various methods and found that the gastric juice, removed from the stomach, would digest meat quite rapidly. He made other interesting and valuable observations regarding the factors influencing the time required for various types of meals to digest and to be expelled from the stomach into the intestinal tract.

In recognition of Dr. Beaumont's classical researches the Thirteenth International Physiological Congress, at its meeting in Boston in August 1929, published a facsimile of the 1833 edition of Dr. Beaumont's book, *Experiments and Observations of the Gastric Juice and the Physiology of Digestion*. A medalion containing the bust of Beaumont was also struck in his memory.

SALIVARY DIGESTION

When food is ingested, the first step in the process of digestion is mastication. The lips, teeth, and tongue all play a part in the process, the object of which is to seize and break the food into small particles which offer a greater surface for subsequent action of digestive juices. The first of these juices with which the food comes in contact is the saliva secreted by the salivary glands and the cells of the mucous membranes of the mouth. There are three pairs of salivary glands, namely, the *parotid glands*, the *sublingual glands*, and the *submaxillary glands*.

Saliva. When food is eaten, the salivary glands are stimulated and saliva pours into the mouth and becomes intimately mixed with the food. For chemical studies human saliva may be collected in large amounts by stimulating salivary activity by chewing paraffin wax. It has been stated that the horse may secrete 84 pounds of saliva in a day, and in the same period an ox may secrete as much as 112 pounds, and man may secrete about 3 pounds.

Human saliva is a semiviscous, colorless liquid with a specific gravity of 1.002 to 1.008. Chemical analysis shows that saliva is composed of water (99.41 per cent) and solids (0.59 per cent). The solids consist of mucin, epithelium, and soluble organic matter (0.35 per cent), and inorganic salts (0.219 per cent). The ash of saliva is rich in potassium and contains the bases sodium, calcium, and magnesium in appreciable quantities. In addition, phosphorus, chlorine, and sulfur are invariably present. Saliva also contains the enzyme *ptyalin* (salivary amylase). The pH of saliva is never far from 7.0, since the phosphates and carbonates act as buffers, keeping the reaction at or near the neutral point.

In man and in many animals saliva functions in two ways; i.e., it acts as a lubricant which assists the process of deglutition (swallowing), and it assists in the digestion of starchy foods. The saliva of carnivora does not possess this property of starch digestion. When saliva is mixed with a viscous solution of starch paste, the solution becomes water-clear and loses its viscosity. Upon treatment of the latter solution with Fehling's

solution, reduction occurs. The original starch fails to give this test. The clear solution does not give a blue color with iodine, indicating that the starch has changed to maltose, glucose, or low molecular weight dextrins which possess sugar-like properties.

The amylolytic enzyme, ptyalin, begins to affect starch hydrolysis just as soon as the finely ground food is thoroughly mixed with the saliva. The thoroughness with which starch digestion takes place in the mouth depends upon the enzymatic activity of the saliva, the length of time the food remains in the mouth, and the fineness of division or thoroughness of chewing.

Most authorities do not attach great importance to the chemical changes that take place in the mouth. The food is swallowed so quickly that salivary digestion does not have time to produce many changes before the food leaves the mouth. Physiological research has shown that the greater part of salivary digestion takes place after the food reaches the stomach and prior to the cessation of salivary enzyme activity, which is inhibited by the accumulation of hydrochloric acid.

STOMACHIC DIGESTION

When the bolus of food, mixed with saliva, is swallowed, it passes through the esophagus or gullet to the stomach where, for a time, salivary digestion continues. Ptyalin, acting on starch, catalyzes the hydrolytic process, with the formation of low molecular weight dextrins, maltose and glucose.

What has been said applies only to those animals possessing a single-sac stomach. The ruminant stomach will be discussed later. The portion of the single-sac stomach which is attached to the esophagus is known as the cardiac portion. The remainder of the stomach is shaped somewhat like a large lop-sided pear. At the other end of the stomach is attached the small intestine. This portion of the stomach is called the *pylorus* or pyloric portion, and it is here that the *pyloric valve* is situated. This valve controls the flow of liquid or semiliquid materials from the stomach to the intestine and prevents the regurgitation of intestinal contents back into the stomach.

The large central portion is known as the *fundus* or fundic por-

tion of the stomach. Most of the *gastric pepsin* (proteolytic enzyme) is thought to be secreted here. The cells producing this enzyme are known as *chief cells*. Other cells, known as *parietal*

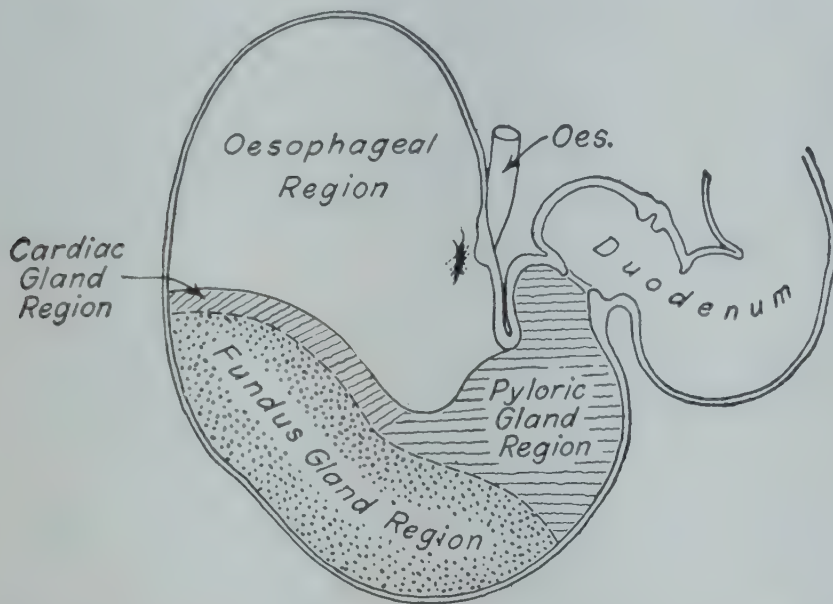


FIG. 12. Diagram of the stomach of a horse. (Courtesy of W. B. Saunders Company.)

cells, are responsible for the production of hydrochloric acid, which causes gastric acidity.

The ruminant stomach

The single-sac stomach is the type possessed by man and such animals as the horse, the hog, and carnivorous animals. Ruminants, such as cattle, sheep, and goats, possess a *compound stomach*, consisting of four divisions or compartments known respectively as (1) the *rumen* or *paunch*, (2) the *reticulum* or *honeycomb*, (3) the *omasum* or *manyplies*, and (4) the *abomasum* or *true stomach*. Only the abomasum is truly comparable with the single stomach cavity of other animals.

The first three compartments of the ruminant stomach are regarded, anatomically and physiologically, as continuations and enlargements of the esophagus in which the feed is fermented and softened preparatory to gastric digestion in the abomasum. In domestic fowls a similar situation exists, the crop being an enlargement of the esophagus, which acts as a storage organ and does not secrete digestive juices.

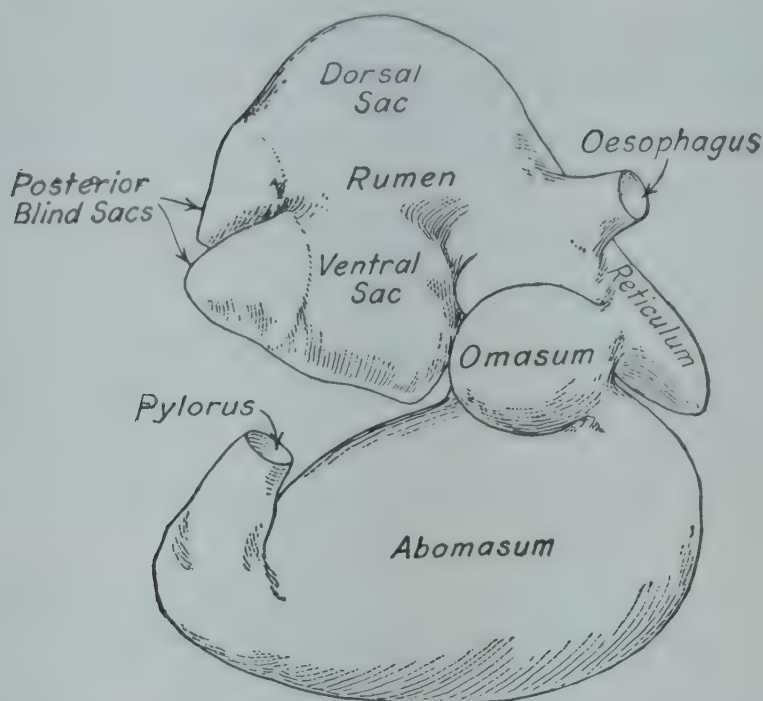


FIG. 13. Ruminant stomach (new-born calf). Right view with rumen raised. (Courtesy of W. B. Saunders Company.)

Rumination. As a rule the ruminant masticates its food very imperfectly at the time of eating. The liquid, soluble, and finely divided portions of the feed pass down the esophagus and through the esophageal canal directly to the true stomach (abomasum). The esophageal canal, which is a continuation of the esophagus, is constructed somewhat like a rubber hose cut lengthwise so that it can be opened. So long as the contents are of a liquid nature, they pass through the closed tube, which remains closed by muscular contraction. However, when large masses of fibrous hay and similar materials are swallowed, the esophageal canal opens and drops the coarse material into the capacious rumen. Here the feed is brought into intimate contact with saliva and various types of enzymes and bacteria (introduced in the feed) which initiate fermentation.

When the ruminant has completed feeding, it has the power to regurgitate and masticate this coarse material at its leisure. This process is known as "chewing the cud." From the rumen, the finely comminuted food passes to the other (grinding) stomachs or compartments and eventually reaches the abomasum where true digestion takes place. During the rumination process, methane, acids, and alcohols are formed in small amounts by fermentation. Certain members of the vitamin B complex (thiamine, riboflavin, pyridoxine, and pantothenic acid) are syn-

thesized in the rumen by microorganisms. This (biosynthesis of vitamins) will be discussed in a subsequent chapter

Stomachic characteristics of the fowl

While we are discussing the stomachic characteristics of the various types of animals, a brief statement should be made

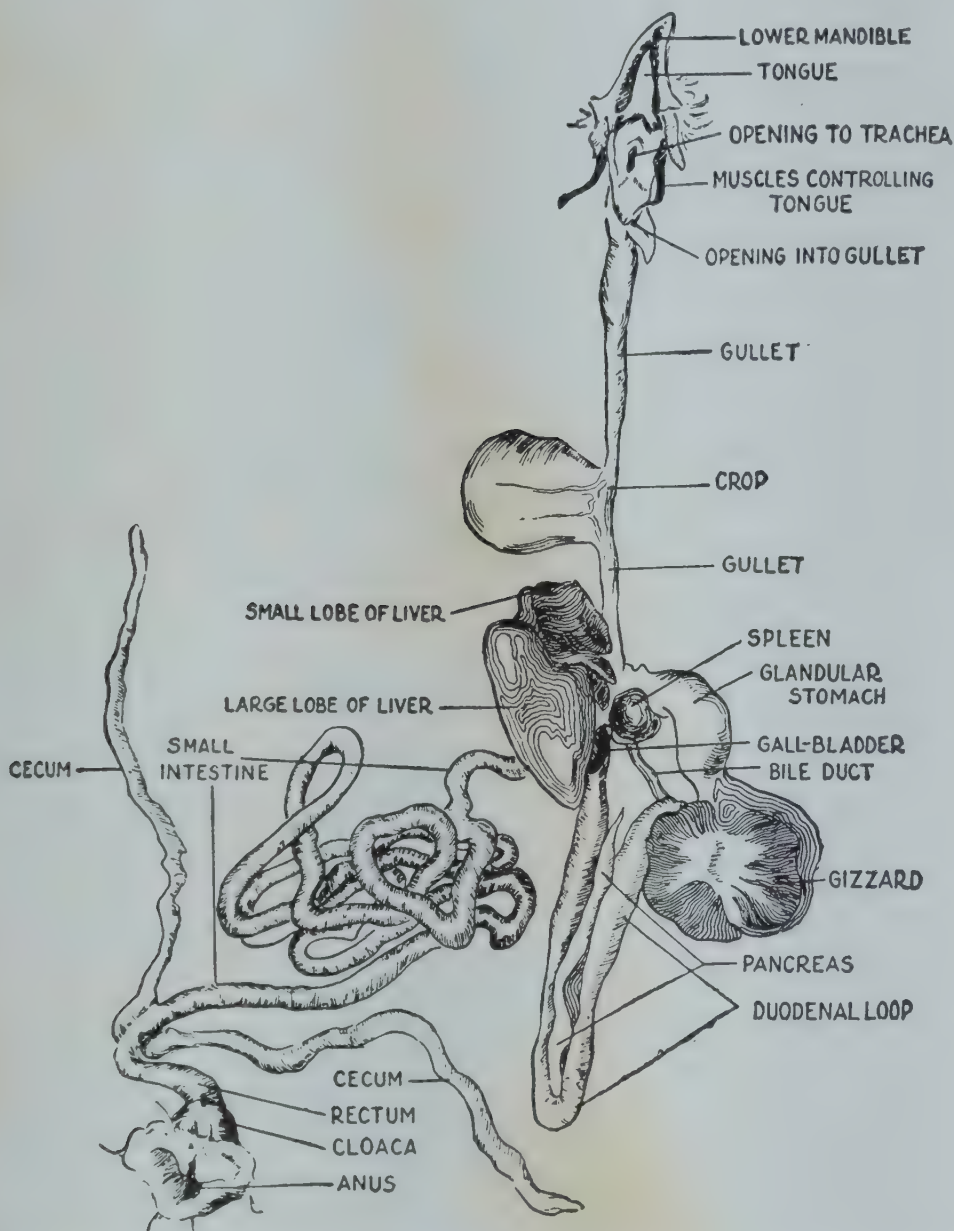


FIG. 14. Showing various portions of the digestive tract of the domestic fowl. (After Lippincott.)

regarding the characteristics of the domestic fowl, for the reason that the structure of its digestive tract differs from that of other animals.

The chicken, like other animals, possesses an esophagus which acts as a transfer organ, but it differs from that of other animals

in that it is divided into two parts, known as the *first* and *second portions*. These are separated by an enlargement of the esophagus which is known as the *crop* and which functions as a storage organ during the hours of feeding. It should be pointed out that no mastication takes place, since fowls do not possess teeth.

From the crop the food passes through the second portion of the esophagus to the *proventriculus*, which is a small expansion of the esophagus, measuring (in the hen) about $1\frac{1}{2}$ inches in length and nearly an inch in diameter. The proventriculus functions primarily as a secretory organ, furnishing gastric juice, but it differs from the true stomach of other animals in that the food does not remain long enough for gastric digestion to take place. From the proventriculus, the food, which has been moistened with gastric juice, passes to the *gizzard*. This large muscular organ, with the aid of grit, grinds the food material to a fine state of division. During the grinding period considerable gastric digestion takes place. The finely ground contents are then passed on to the small intestine.

Gastric digestion

We now return to a more detailed discussion of gastric digestion in animals possessing the single-sac stomach. The changes that are typical for humans and for animals such as the horse, the hog, the dog and the cat apply in a large measure to other animals.

When the bolus of food reaches the cardiac portion of the stomach, the latter, because of its collapsible nature, becomes completely filled with food. As additional food is swallowed, the successive portions find the stomach walls occupied. As a result later food additions are received into the interior of the stomach, while some of the food remains at the cardiac portion, near the esophagus. As the muscular movements (peristalsis) occur, there is a tendency for the food to become mixed. However, since this does not occur immediately, the gastric juice secreted by the cells in the stomach wall cannot mix with the interior portion for an appreciable time. Consequently salivary digestion continues in the stomach for various lengths of time,

until it is stopped by the accumulated acid of the gastric juice. The foregoing statements explain why salivary digestion in the stomach is considered to be of greater importance than salivary digestion in the mouth. At this point varying quantities of starch have been changed to the low molecular weight dextrins, maltose and glucose, although relatively large amounts of starch may still remain unchanged. During the process just described the gastric juice is increasing in volume and the gastric contents are becoming more thoroughly mixed with the gastric juice, which initiates other chemical changes to be discussed in a subsequent paragraph.

Gastric juice. Normal gastric juice is a thin, colorless liquid, acid in reaction, and possessing a specific gravity of 1.001 to 1.010. The solid matter comprises about 0.5 per cent and is composed principally of sodium chloride, potassium chloride, phosphates, and the enzyme, *pepsin*. The acidity of gastric juice is caused by free hydrochloric acid, which has the power to destroy the amylolytic activity of the salivary enzyme, ptyalin, but which is necessary for the maximum activity of the gastric protease, pepsin. The normal acidity of gastric juice in man is about 0.4 to 0.5 per cent. Proteins and other substances may combine with hydrochloric acid, lowering gastric acidity. This is differentiated from free hydrochloric acid and is called *combined acid*. The antiseptic action of gastric juice, which normally prevents fermentation in the stomach, is thought to be due to hydrochloric acid.

Pepsin is the only important enzyme of the gastric juice. It is not secreted as active pepsin but is first produced in an inactive form called *pepsinogen*. This zymogen is the precursor or mother substance of pepsin. When pepsinogen comes in contact with hydrochloric acid (which acts as an activator) active pepsin is formed. Pepsin is most active in acid solutions, and it has been shown that the proteolytic activity of pepsin is not due to hydrochloric acid, per se, for many acids, at proper concentration, will produce satisfactory results. The concentration of hydrochloric acid at which pepsin seems to act best ranges from 0.1 to 0.25 (*pH* 1.5 to 2.0) per cent. Pepsin is inactivated at *pH* 6 or higher. Like many enzymes of animal origin pepsin is very active at temperatures from 38 to 40° C.

Gastric rennin. This is a protein-coagulating enzyme which has the property of curdling milk, thereby holding the milk proteins in the stomach until peptic digestion can take place. According to Van Slyke and Bosworth, rennin owes its curdling or coagulating action to the formation of paracasein which combines with calcium, forming insoluble calcium paracaseinate. If calcium salts are removed from milk by addition of oxalate, rennin will not form the insoluble paracaseinate. This experiment shows that calcium salts are necessary for the formation of curds by rennin. Pepsin also possesses the power to curdle milk, although its activity is less than that of rennin. Although rennin is found in the stomachs of infants and young animals, such as the calf, there is little, if any, rennin secreted by adult humans or mature animals. Hydrochloric acid and pepsin probably play the major role in gastric coagulation of milk so far as adult human beings are concerned. Rennin acts best in slightly acid solutions (pH 6.0 to 6.5), although it will coagulate casein in neutral and even slightly alkaline solutions. Commercial rennin, known as *rennet*, is obtained from the calf stomach and is used in creamery practice in the manufacture of cheese.

When casein is made for industrial purposes, it is precipitated from skim milk by adding acid. At the isoelectric point (pH 4.6 to 4.8) casein precipitates. This precipitate consists of casein, as such, and differs from calcium paracaseinate, which is of smaller molecular size.

Gastric secretion. In terminating our brief discussion of gastric juice our mental picture of gastric secretion would not be complete without reference to the work of Edkins, and Koch and co-workers. These scientists believe that the tissues contain a chemical hormone called *gastrin*, which stimulates gastric secretion. The presence of food liberates gastrin, which, in turn, stimulates the flow of gastric juice. This hormone can be extracted, and, when it is injected into other animals, gastric secretion occurs almost immediately. It does not affect salivary secretion. Chemical investigations indicate that it is probably related to, if not identical with, *histamine*.

Chemical changes in gastric digestion. We have discussed the changes through which starches may pass in early gastric diges-

tion, owing to the continued action of salivary amylase. We shall now turn our attention to the changes occurring in foods as the result of true gastric digestion.

So far as chemical changes are concerned, gastric digestion may be considered primarily proteolytic in nature. In other words, the main function of the gastric juice is to digest protein materials. Pepsin, which is responsible for the hydrolytic catalysis of proteins, splits the protein molecules to proteoses and peptones. Some peptides and amino acids are found in the digestion mixture, but the bulk of the hydrolyzate is composed of peptones, which are soluble in water.

Recent researches indicate that pepsin activity depends on the presence and position of the amino acids, tyrosine and phenylalanine, in the protein molecule. Proteins that do not possess peptide linkages involving these amino acids are not readily hydrolyzed by pepsin. Simple sugars do not seem to increase during true gastric digestion, nor does there appear to be appreciable fat hydrolysis. Some inversion of sucrose may be brought about by the action of HCl during gastric digestion. It will be seen, therefore, that the acid chyme (liquified stomach contents) may consist of proteins, fats, and carbohydrates which have resisted digestion and, in addition, proteoses, peptones, peptides, amino acids, and simple sugars.

INTESTINAL DIGESTION

When the chyme has reached the proper state of fluidity and acidity, the pyloric valve opens intermittently and allows the stomach contents to flow into the duodenum. This is the portion of the small intestine (about 11 inches long in man) which is attached to the pyloric end of the stomach. The first spurt of acid chyme, as it comes in contact with the intestinal mucosa, causes the secretion of duodenal juice, an albuminous secretion with an alkaline reaction. In the duodenal juice is a heat-labile substance called *enterokinase*, which activates the proteinase of pancreatic juice. Three digestive juices play an important role in intestinal digestion, namely, *pancreatic juice*, *bile*, and *intestinal juice*. These will be discussed in the order named.

Pancreatic secretion. Lying along the duodenum is a most important organ, the pancreas, which pours its secretion (pancreatic juice) into the intestine. The pancreas is a rather small tubular gland which delivers its digestive juice into the intestine by intermittent rather than by continuous flow. The discovery by Bayliss and Starling of the mechanism of pancreatic secretion is one of the classical discoveries of modern physiology. These workers proved that the mucosa of the duodenum contains a chemical hormone, which they named *secretin*. The introduction of acid chyme from the stomach releases the hormone, which, by way of the blood stream, stimulates the pancreatic gland to pour forth its digestive juice. The release of secretin from the intestinal tissue is brought about, in all probability, by the contact of the acid stomach contents with the wall of the intestine. The basis for this statement lies in the fact that maceration of duodenal tissue with hydrochloric acid produces an extract which stimulates the flow of pancreatic juice. When this extract is injected into the blood stream of another animal, the flow of pancreatic juice follows immediately. When intestinal extract that has not been treated with HCl is injected, no pancreatic stimulation can be noted. Furthermore acid extracts of other organs do not have this effect. Further proof of the transfer of secretin by the blood was shown by a most ingenious experiment. The blood streams of two dogs were connected in such a manner that they possessed one blood-circulatory system. Tubes were placed in the pancreatic duct of both animals, and a dilute acid solution was introduced into the duodenum of one of the dogs. The effect of the acid was to cause pancreatic secretion simultaneously in both dogs—proving that secretion of secretin took place in one dog and was transferred by the blood to the other animal. Hammarsten and Ivy have isolated secretin in crystalline form. It contains about 8 per cent nitrogen and has the properties of a peptide. The existence of another hormonelike substance called *pancreozymin* has been postulated. According to some workers the hormone secretin stimulates the flow of fluid and bicarbonate, whereas pancreozymin stimulates the production of pancreatic enzymes.

Pancreatic juice is a clear alkaline liquid which coagulates on heating. The alkalinity is due to the presence of sodium bi-

carbonate, and the coagulable portion consists of protein which, in some cases, is of sufficient quantity to form a solid coagulum on heating.

Enzymes of the pancreatic juice. Pancreatic juice owes its digestive properties to three types of enzymes, namely, those which (1) act on proteins and protein degradation products, (2) digest starches, and (3) hydrolyze fats. In other words, pancreatic juice contains *proteases*, *amylases*, and *lipases*. When pancreatic juice is allowed to flow directly into a receptacle without coming in contact with the intestinal juices, it possesses no protein-splitting properties. If, however, the pancreatic juice is allowed to flow into the intestine and is then removed, it is found to be highly proteolytic. This has been explained by the presence in the intestinal juice of an activator called *enterokinase*, which activates the proenzyme, *trypsinogen*, forming active *trypsin*. In other words, the enzyme trypsin is secreted by the pancreas in inactive form, from which it is changed to trypsin by contact with enterokinase. If the intestinal juice is boiled prior to mixing, trypsin is not formed, showing that enterokinase is heat-labile. The mechanism of this activation is far from clear. Evidence points to the fact that, once a small amount of trypsin is formed, the process becomes *autocatalytic*; that is, additional supplies of enterokinase are not required for the activation of trypsinogen.

Pancreatic juice furnishes three other enzymes that have to do with protein digestion, namely, *chymotrypsin*, *carboxypolypeptidase*, and *aminopolypeptidase*. *Chymotrypsin* exists in the pancreatic gland as an inactive proenzyme known as *chymotrypsinogen*. This proenzyme possesses no protein-splitting properties until it becomes activated by contact with trypsin. Trypsin and chymotrypsin do not hydrolyze proteins beyond the proteose or polypeptide stage. The latter are hydrolyzed by *carboxypolypeptidase* and *aminopolypeptidase*, which are also present in the pancreatic juice. Carboxypolypeptidase acts only on those polypeptides with free carboxyl groups, whereas aminopolypeptidase attacks those polypeptides with free amino groups.

The starch-splitting properties of pancreatic juice are due to the presence of the amylase, *amyllopsin*. When the juice is boiled the amylolytic properties are destroyed. Carnivora, which

possess no salivary ptyalin, must depend upon amylopsin to digest any starches that may be ingested in the food. This enzyme, like ptyalin, hydrolyzes starches and dextrins to the maltose stage, at which point maltase must be present if the maltose is to be hydrolyzed to simple sugars.

Pancreatic juice not only assists in the emulsification of fats and oils, but it also contains a very active lipase called *steapsin*, which hydrolyzes the glycerides to glycerol and fatty acids. If pancreatic juice is boiled, these properties are lost. In the presence of bile, which will be discussed in a succeeding paragraph, steapsin acts much more rapidly and efficiently, hydrolyzing larger amounts of fat per unit of time.

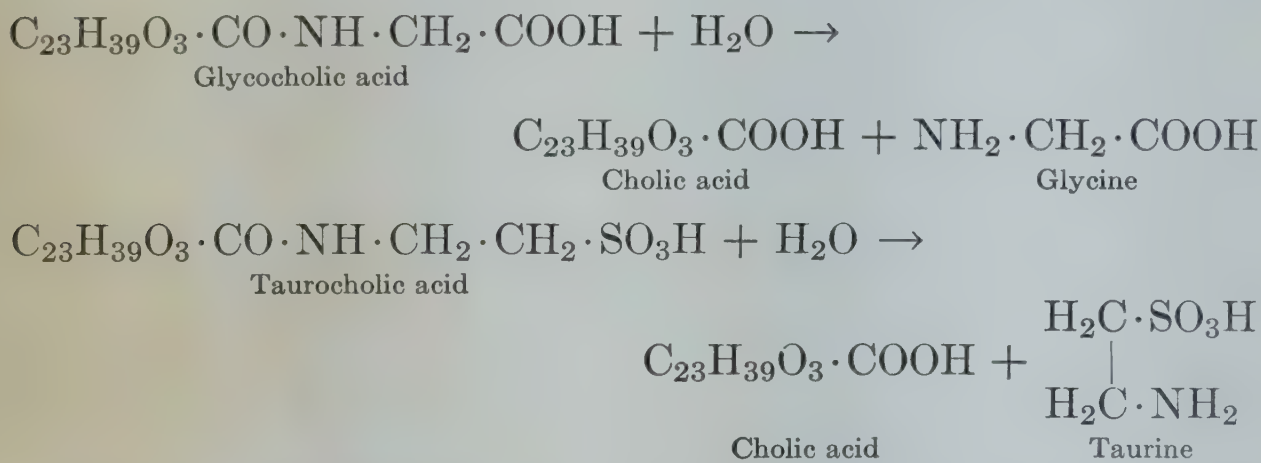
There is no good evidence that steapsin exists as a proenzyme. Observed stimulation of lipase activity in the presence of bile salts and other intestinal substances can be explained more satisfactorily on the basis of lowered surface tension, increased emulsification of fats, and increased opportunity for steapsin to come in intimate contact with the surfaces of the globules of emulsified fats.

Bile. The second of the juices assisting in intestinal digestion is *bile*, or gall, a bitter fluid secreted into the intestine by the liver. It may be green in color when fresh, but usually it is yellowish brown. It is extremely bitter to the taste, possesses a specific gravity of about 1.020, and is characterized by containing bile pigments, bile salts, and the alcohol, cholesterol. In the horse the flow of bile is continuous, but in man and in animals such as cattle, sheep, and swine the bile is stored in the gall bladder, from which it pours, intermittently, through the bile duct to the duodenum. In man the opening of the bile duct is very close to that of the pancreatic duct, which facilitates the intimate mixing of these important digestive fluids. In carnivora the juices are delivered to the intestine through a common duct, whereas in herbivora the ducts are separate and somewhat removed one from the other.

The flow of bile is caused by contraction of the gall bladder. This contraction is stimulated by free fatty acids formed during fat hydrolysis. Another regulatory substance which assists in regulating the emptying of the gall bladder is a hormone called *cholecystokin**in*, which originates in the intestinal mucosa.

Bile acids. Bile does not possess digestive enzymes but plays a most important part in fat digestion. Because of its alkalinity and the presence of *bile salts* (esters of cholic acid with taurine and glycine), bile assists in the emulsification of fats. In addition the free fatty acids combine with the bile salts, in which form they seem to be able to pass through the intestinal wall. Many writers regard bile as nature's laxative, since it hastens peristalsis and absorption. By so doing the chances for putrefaction of foods in the intestine are reduced to the minimum.

Taurocholic acid and glycocholic acids are complicated esters which yield the following products on hydrolysis:



It will be noted that the structure of taurine is closely related to that of the amino acids, cysteine and methionine.

Bile pigments. Bile contains four pigments, namely, *bilirubin*, *biliverdin*, *stercobilin*, and *urobilin*. The principal pigment of the body is the *hemoglobin* of the blood. As red blood cells die, hemoglobin is liberated. This red pigment splits to form an iron-containing substance called *heme* and a protein called *globin*. The heme breaks down to form protoporphyrin and liberates iron for the formation of new hemoglobin. The protoporphyrin is converted into the yellow bile pigment, bilirubin. Biliverdin, a green bile pigment, is formed from bilirubin by oxidation. When bilirubin undergoes reduction, a brown pigment, known as stercobilin, is formed. The latter gives feces their characteristic brown color. Urobilin also originates from bilirubin. On further reduction, this pigment forms *urobiligen*. The yellow color of urine is due to the presence of urobilin and urobiligen. The term urochrome is often used to refer to the yellow urinary pigments.

Intestinal juice. This digestive juice is secreted by the glands lining the walls of the small intestine. Enzymes secreted in the intestinal juice are the disaccharide-splitting enzymes, *sucrase*, *maltase*, and *lactase*; *peptidases*, which hydrolyze peptides to amino acids; and *phosphatase*, which has the ability to split phosphoric acid from hexose phosphates, nucleotides, and glycerophosphates. Until recent years the existence of an enzyme called erepsin was thought to split proteoses and peptones to amino acids. It is now known that the so-called "erepsin action" is due to the presence of *aminopolypeptidase*, *carboxypolypeptidase*, and *dipeptidase*. The first two of these enzymes hydrolyze polypeptides to simpler peptides and amino acids, and the latter hydrolyzes dipeptides to amino acids. In other words, these enzymes do not act on native food proteins but complete the hydrolytic changes on polypeptides resulting from gastric and pancreatic digestion.

Another important substance in intestinal juice is *enterokinase*, which has the property of activating trypsinogen of the pancreatic juice to form the active proteolytic enzyme, trypsin. Other enzymes in intestinal juice are *nuclease*, which splits nucleic acid into nucleotides; *nucleotidase*, which splits nucleotides into nucleosides and H_3PO_4 ; and *nucleosidase*, which splits nucleosides into sugars and purines or pyrimidines. As their names indicate, the carbohydrases of intestinal juice, *sucrase*, *maltase*, and *lactase*, hydrolyze sucrose, maltose, and lactose, respectively.

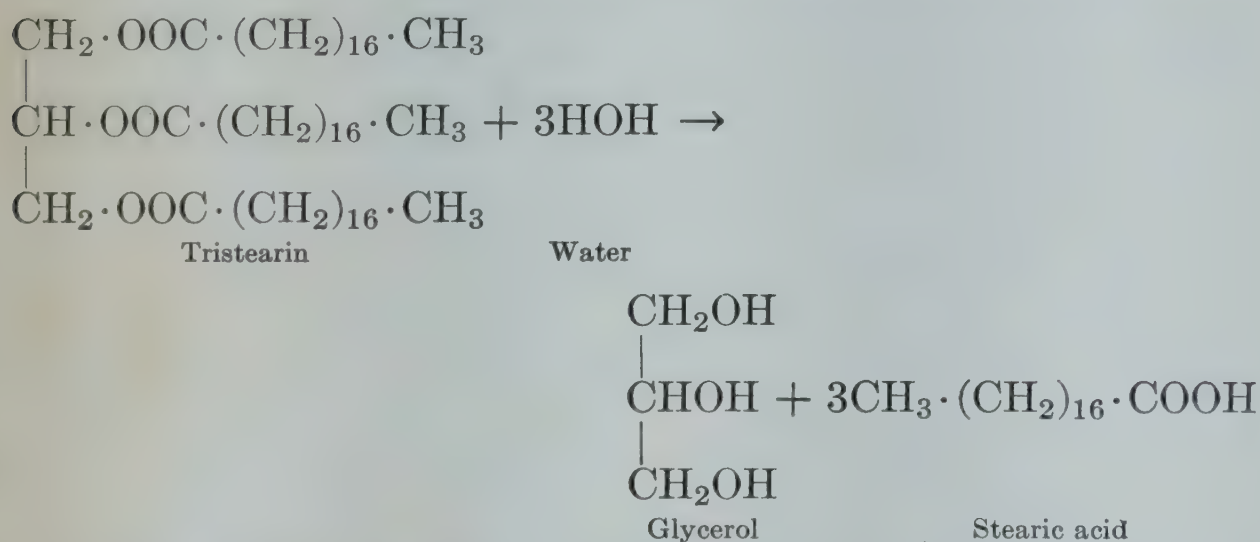
Chemical changes in intestinal digestion. When the acid chyme is introduced into the duodenum, some of the proteins, fats, and carbohydrates are unchanged, and some are incompletely hydrolyzed. Since nature strives to reduce these substances to the simplest and most soluble compounds, it is to be expected that intestinal digestion should finish what salivary and gastric digestion have started and initiate those changes that have not yet taken place.

The acid of the chyme is gradually neutralized by the alkaline intestinal contents, and the mass is mixed and passed on down the intestine by peristaltic muscular contractions which flow up and down the intestine. The pancreatic, bile, and intestinal juices become mixed with the food, and proteins, fats, and carbo-

hydrates are hydrolyzed and prepared for absorption by the body.

Proteins. Proteins that have escaped gastric digestion are hydrolyzed to proteoses, peptones, and polypeptides by trypsin and chymotrypsin. Carboxypolypeptidase attacks the polypeptides by removing the amino acids containing free carboxyl groups, and the amino polypeptidases attack those polypeptides containing free amino groups. Thus it is to be noted that the eventual goal of proteolytic changes is to form soluble amino acids which can be absorbed and utilized.

Fats. *Steapsin*, pancreatic lipase, splits the glycerides to glycerol and free fatty acids, according to the following equation:



Carbohydrates. Starch and dextrans, which may have escaped hydrolysis in the stomach, are acted upon by amylopsin (pancreatic amylase), and the major portion of these polysaccharide molecules is changed to maltose. Maltose, sucrose, and lactose are hydrolyzed to simple sugars by maltase, sucrase, and lactase, respectively.

During digestion of straw and hay by the ruminant and the horse, polysaccharides are broken down to glucose, fructose, and pentose sugars by the fermentive action of microorganisms. Methane, organic acids, and alcohols are also by-products of these fermentive processes.

Divisions of the intestinal tract. The small intestine is a rather narrow tube which starts at the stomach and ends at the cecum. At this latter point is a valve, known as the *ileocecal valve*, through which the intestinal contents may pass to the cecum. The small intestine is divided into three parts: (1) the

duodenum, (2) the *jejunum*, and (3) the *ileum*. The length of the small intestine averages, in the horse, about nine times that of the body; in the ox and sheep, sixteen times; and in the hog, eleven times.

The *cecum* is an enlargement of the alimentary canal and serves as a storage organ for further digestion and fermentation. No digestive juices are secreted here, but the organ is of great importance in spite of this fact. In a way it might be compared with the rumen or paunch of ruminants, although it differs from the rumen in that digestive changes take place in the cecum, owing to the presence of enzymes which originated in the small intestine. The small intestine opens into the cecum, and not far from the intestinal inlet is another tube, somewhat larger, from which the cecal contents pass to the large intestine. This large outlet tube is called the *colon*, and just before it leaves the body it enlarges and becomes the *rectum*. Together, the colon and rectum are known as the large intestine.

Armsby states that, in a general way, the size of the cecum is inversely proportional to that of the stomach. In the horse the cecum is very large and holds about 16 per cent of the total contents of the digestive tract. This organ allows the food to remain for long periods of time in contact with digestive juices which have come from the small intestine, and, as a result of fermentive and putrefactive changes, the horse is able to digest and utilize crude fiber to a very appreciable extent. The cecum in ruminants is relatively small, with a capacity of about 3 per cent of the tract. That of hogs is not much higher, being about 5 per cent.

In the horse the large intestine is also highly developed and may constitute as high as 45 per cent of the total capacity of the digestive tract; like the cecum, it serves as a storage organ in which the hydrolysis of difficultly digestible foods continues. In the domestic fowl two ceca connect with the intestine at the same point, forming a Y-shaped structure. (See Fig. 14.)

Absorption. The small intestine is lined with folds of tissue containing thousands of tiny fingerlike projections known as *villi*, which increase the absorptive surface of the intestine enormously. It was thought at one time that proteins were absorbed by the intestines in the form of peptones. This idea

has been abandoned as the result of work by Folin and Denis, Van Slyke and co-workers, and Abel and others. The evidence now points to the fact that proteins are absorbed in the form of amino acids. These are absorbed in the small intestine and are either built into tissue or deaminized in the liver and the nitrogen excreted as urea. These metabolic phases will be discussed in Chapter 22.

At this point it might be well to emphasize that the villi occur in largest amount in the duodenum. Absorption occurs in the jejunum and ileum in smaller amount per unit of length, but, on account of the fact that these portions of the intestine are much larger than the duodenum and possess more surface, it seems reasonable to believe that a greater portion of the absorption takes place in the jejunum and ileum.

The villi are supplied with two circulatory systems: (1) a blood system consisting of arterial and venous networks, and (2) a lacteal or single lymphatic capillary which communicates with the lymphatic system. From the villi, absorbed nutrients are carried via the mesenteric veins to the portal vein and thence to the liver and body tissues. A major portion of the fats, however, are absorbed in the lacteals and reach the tissues via the lymphatic system, a rather poorly defined circulatory system, containing a fluid called *lymph*, which will be discussed in more detail in Chapter 17.

In our discussion of the digestion of fats, we learned that bile salts assist in fat digestion. These bile salts also seem to be essential for the efficient absorption of fatty acids. Glycerol, a normal product of fat hydrolysis, is water-soluble and is readily absorbed. Fatty acids, however, are not water-soluble and cannot be absorbed as free acids. Consequently they form a soluble complex with bile salts, in which form they are able to diffuse through the intestinal membrane. This bile salt complex undergoes degradation as soon as it is absorbed, and tissue lipases seem to possess the power of resynthesizing glycerides from the absorbed glycerol and the free fatty acids released from the bile salt-fatty acid complex. The bile salts eventually reach the liver, where they again become normal bile constituents.

Although the fatty acid-bile salt complex explanation for fat absorption is generally accepted, there is good reason to believe that some unhydrolyzed fat is absorbed in highly emulsified form, with globules so small that they can pass through the intestinal mucosa. In fact, Baldwin of England goes so far as to state that much, if not most, of the fat absorbed during digestion may be absorbed as finely emulsified unhydrolyzed glycerides.

As we have suggested in a previous paragraph, a large proportion of the fat is shunted around the liver by being carried to the tissues via the lymphatic system. This fat-rich lymph is carried by the thoracic duct, where it is poured into the blood stream at the junction of the jugular and subclavian veins. This creamy lymph is called *chyle*.

Carbohydrates are absorbed by the villi capillaries in the form of glucose, fructose, and galactose, and these simple sugars are carried by the blood stream to the liver where they are deposited as glycogen (animal starch), or they are sent to the tissues where they are utilized or deposited as glycogen. Some disaccharide absorption may take place, but it is considered of minor importance.

Although glucose, fructose, and galactose may be deposited in the liver and muscles as glycogen, it should be noted that, when tissue glycogen undergoes hydrolysis to form sugar for metabolic purposes, the only sugar formed is glucose. Lactose, because of its relatively low solubility, is not utilized so efficiently as glucose and fructose. As a result an appreciable amount of lactose finds its way to the lower intestine where it often plays an important role in increasing the lactic acid-forming organisms of the intestinal flora. These organisms tend to discourage the growth of putrefactive organisms in the intestine.

Absorption in the large intestine. After the products of digestion have been absorbed in the small intestine, the unabsorbed mass passes into the large intestine. The digestive changes that take place here are not of great importance. The mucous membrane of the large intestine contains gland cells, but the secretion is largely mucus, and no digestive enzymes are secreted. The chemical changes that take place here are due largely to bacteria, but it is possible that enzymes may be carried through

from the small intestine, and some hydrolytic changes may occur. Like the secretions in the small intestine the secretions in the large intestine are usually alkaline. Putrefaction is a normal process, resulting in the production of proteoses, peptones, amino acids, and other decomposition products such as indol, skatol, and ammonia. In addition to the putrefactive changes, carbohydrates, such as cellulose, are acted upon by fermentive organisms, leaving behind lactic, butyric, and acetic acids, alcohols, and gases such as methane, carbon dioxide, and hydrogen.

Water is absorbed in the large intestine, and the undigested mass gradually takes on the characteristics of feces. Feces are composed mainly of undigested food materials such as cellulose, keratin, and other proteins, bacteria, and fats. In addition to these are found the digested nutrients that have escaped absorption. Metabolic products such as indol and skatol are present, and it is to these substances that the odor of feces is largely due.

Detoxication. It has been estimated that nearly one-third of the solid matter of normal feces consists of bacteria. Bacterial flora of the intestine are responsible for a variety of chemical reactions which give rise to a number of chemical compounds. Some of these compounds are harmless or even beneficial, whereas other compounds are definitely toxic.

Bacterial activity, which results in the production of such compounds as acetic, butyric, and lactic acids, or ethyl alcohol, is beneficial because the body can absorb and utilize these compounds. Under proper conditions, microorganisms are capable of synthesizing appreciable amounts of such vitamins as thiamine, riboflavin, folic acid, and vitamin K. Biosynthesis of vitamins has been shown to take place in the paunch of ruminants and in the digestive tract of rats and humans.

The end products of carbohydrate breakdown in the intestine are usually harmless, whereas many products resulting from protein breakdown are distinctly toxic. Many of the ill effects due to constipation have been attributed to the absorption of these toxic products, although medical authorities are not in complete agreement on this point. Nevertheless it is well known that intestinal putrefaction does produce products that are highly potent and that the body possesses ways and means of

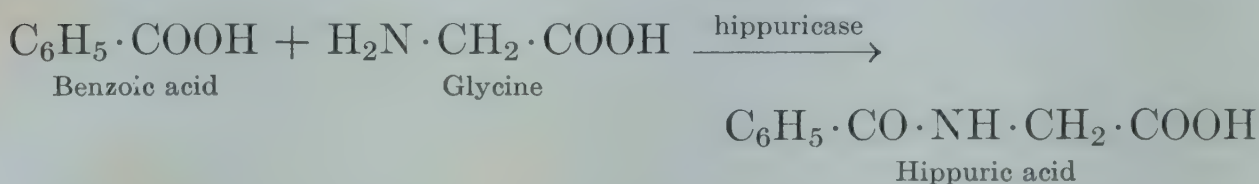
protecting itself against these toxic materials. These defense mechanisms are known as *detoxication mechanisms*.

The toxic products produced by intestinal putrefaction consist almost entirely of substances formed by the action of bacteria on amino acids derived from proteins. If amino acids are decarboxylated, amines are formed. The following amines are known to arise from the amino acids listed below:

AMINO ACID	AMINE FORMED
Arginine	Putrescine (tetramethylenediamine)
Lysine	Cadaverine (pentamethylenediamine)
Phenylalanine	Phenylethylamine
Tyrosine	Tyramine (hydroxyphenylethylamine)
Tryptophan	Indolethylamine
Histidine	Histamine (imidazolethylamine)

Histamine is also formed in the tissues, and we have already learned that this compound functions as a hormone in stimulating gastric acidity. In anaphylactic shock, histamine is found in the blood in relatively large amounts. The body attempts to protect itself against excessive histamine toxicity by means of an enzyme, *histaminase*, found in the intestine. The liver is of prime importance in detoxication, destroying many toxic compounds. The kidney also inactivates toxic compounds through the agency of enzyme systems.

Benzoic acid, another toxic product resulting from intestinal putrefaction, is detoxicated by uniting with glycine to form a harmless excretory product, *hippuric acid*.



Thus it can be seen that glycine serves as a detoxicating agent. Likewise, toxic phenols, which are formed in intestinal putrefaction, are excreted as harmless esters of sulfuric acid or in combination with glucuronic acid.

In other words, the body has a number of protective mechanisms for detoxicating harmful products formed in the body. These include reductions, hydrolyses, oxidations, and the neutral-

ization of toxic compounds by chemical synthesis of harmless compounds which can be excreted. In the next chapter we shall consider some of the chemical characteristics of some of the more important body tissues that are constructed from the nutrients which have just been discussed.

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17 • The Chemistry of Blood, Lymph, and Body Tissues

BLOOD AND LYMPH

Blood. The principal functions of the blood are (1) to carry nutrients from the digestive tract to the body tissues, (2) to carry away waste products formed during metabolism, (3) to transfer oxygen and carbon dioxide between lungs and tissues, (4) to distribute products of internal secretion (hormones), (5) to assist in the control of the water content of tissues, (6) to maintain proper pH and body temperature, and (7) to serve as a defense against disease. It should be noted that, although blood is sometimes called a “circulating” or “liquid tissue,” it differs from many other body tissues in that no metabolic activity of importance occurs in the blood.

When blood is separated by centrifuging, we find that approximately 40 per cent of the blood can be segregated in the form of *blood corpuscles*, and the remaining 60 per cent consists of a straw-colored liquid, known as *plasma*. The corpuscular fraction consists almost wholly of red blood cells (*erythrocytes*). Other solid constituents are white blood cells (*leucocytes*) and *blood platelets*.

General properties of blood. The average specific gravity of normal human blood varies from 1.055 to 1.060. There is a tendency for the specific gravity of blood to be lower during sleep than during waking hours, and it is increased by exercise and decreased slightly after meals. Individual variations are quite marked. The specific gravity of red and white cells is

greater than that of plasma, and the erythrocytes settle out faster than the leucocytes, since the former are heavier. The amount of blood in the body varies with different species of animals. On the basis of percentage of body weight the amount of blood in swine varies from 2.5 to 5.0 per cent, man has about 5.0 per cent, fowls from 7.7 to 10.0 per cent, and the horse about 10 per cent.

The osmotic pressure of human blood is equivalent to that of a 0.9 per cent solution of NaCl. The latter is known as *physiological saline solution* and is sometimes used for intravenous injections. *Ringer's solution* is preferred to physiological saline because it contains a better mixture of cations. Ringer's solution for mammals consists of NaCl (0.90 per cent), CaCl_2 (0.26 per cent), and KCl (0.03 per cent). Blood produces a slightly alkaline reaction with litmus paper, although the pH is always close to 7.0.

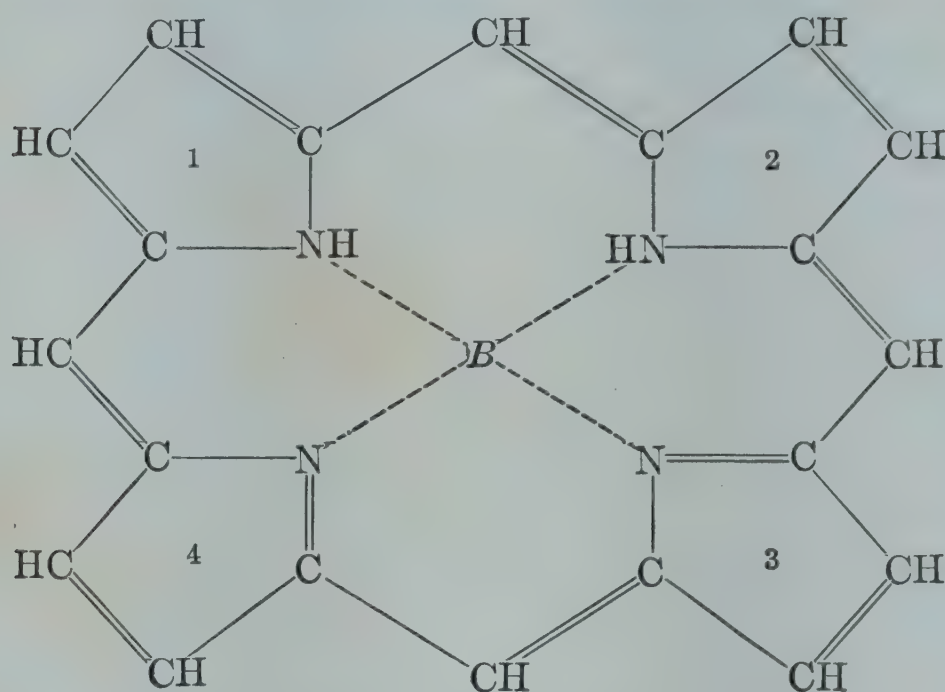
Erythrocytes. The erythrocyte, or red cell, count varies in different types of animals. In men the average number is about 5,000,000 per cubic millimeter, whereas the blood of women will average about 4,500,000. The chief function of erythrocytes is the transference of oxygen. Hemoglobin, the most important constituent of red cells, is responsible for oxygen transfer. When the erythrocytes are ruptured, or if the permeability of the cell membranes is increased, the red hemoglobin forms an homogeneous red solution and the blood is said to be *laked* or *hemolyzed*.

Erythrocytes, which are non-nucleated, highly specialized cells, originate in the bone marrow and spleen, although they may originate in the liver or lymph nodes under certain abnormal conditions. Erythrocytes undergo continuous disintegration in the blood vessel walls of the liver, giving rise to bile pigments, which are formed from the pigment, *heme*.

Hemoglobin. This red substance is a conjugated protein consisting of the iron-containing pigment, *heme*, and a protein, *globin*. The hemoglobin content of normal human blood averages about 15.0 per cent. Diagnostic measurements are usually expressed in terms of *per cent of normal*, in which the per cent of normal hemoglobin is divided by the per cent of the normal red cell count. When a person is anemic owing to a reduction in the number of erythrocytes or to a reduction in the amount

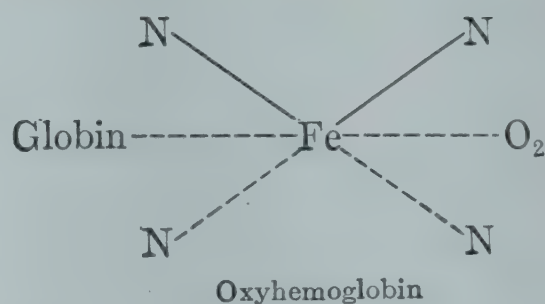
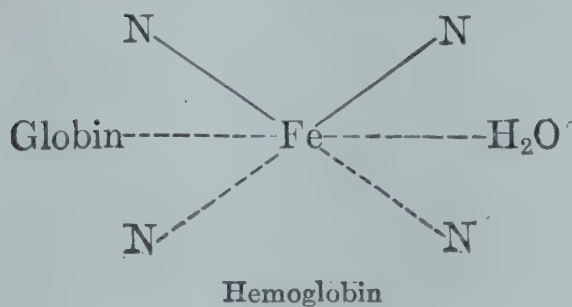
of hemoglobin in the cells (or both), the calculated per cent of normal will indicate the severity of the disease.

The plant pigment, chlorophyll, and the animal pigment, hemoglobin, are closely related chemically. Both pigments contain a pyrrole-containing nucleus, called *porphin*. When chemical groups are attached to this nucleus, the derivatives are usually called *porphyrins*. For example, protoporphyrin, the mother substance of heme, contains the porphin nucleus to which methyl and ethylene radicals are attached to rings 1 and 2, respectively; rings 3 and 4 contain substituted propionic acid molecules.



Porphin nucleus containing four pyrrole rings

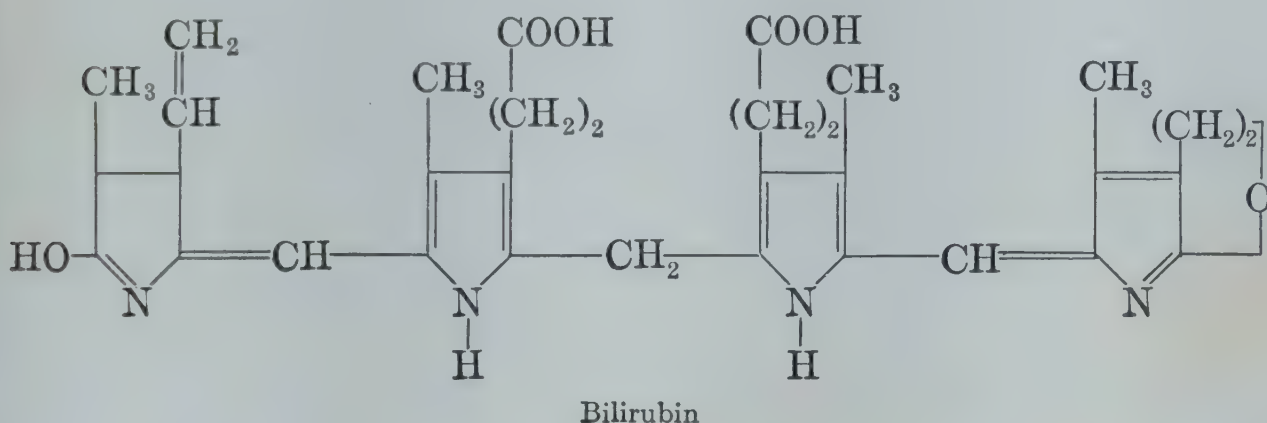
In the chlorophylls magnesium is combined with the pyrrole nitrogen at point *B*, whereas iron occupies this position in the porphin nucleus in hemoglobin. In hemoglobin and oxyhemoglobin the iron is present as ferrous iron. Oxygen is carried to the tissues in the form of *oxyhemoglobin*.



When the protein globin is removed, iron-containing heme remains. When heme undergoes degradation, the iron is avail-

able for the formation of new hemoglobin and the protoporphyrin becomes available for formation of the bile pigments, *bilirubin* and *biliverdin*.

Bilirubin, the parent substance of the bile pigments, has the following structure:



Leucocytes. White blood cells (leucocytes) are normal constituents of blood. In human blood the normal white cell count may fall as low as 5000 per cubic millimeter, and maximal normal counts of 10,000 are not uncommon. The average white cell count is about 7000 per cubic millimeter of blood.

Under the microscope these cells are present in many characteristic forms. One type consists of *small* and *large lymphocytes*, which reach the blood via the lymphatic system. Others are described as *large mononuclear*, *transitional*, and *polymorphonuclear*, depending on the shape and appearance of the cell nuclei. Others are classified according to their staining characteristics. For example, *eosinophiles* are stained red with eosin, whereas *basophiles* are stained a blue color with methylene blue.

The polymorphonuclear leucocytes constitute about 65 to 70 per cent of the white cells of the blood. They possess *amoeboid movement* and have the ability to *engulf and destroy bacteria* and foreign substances in blood. This process is known as *phagocytosis*, and the amoeboid white cells are known as *phagocytes*. Consequently leucocytes function to combat infections. In acute infections the white cell count tends to increase markedly. In acute appendicitis, for example, the white cell count may be as high as 15,000 to 20,000 per cubic millimeter of blood. This is used as a routine method to assist the physician and surgeon in diagnosing the disease.

Plasma and serum. When the blood corpuscles are removed from normal, unclotted blood, the remaining straw-colored liquid is called plasma. Although, from a quantitative standpoint, hemoglobin is the most important protein in whole blood, the most important plasma protein is *fibrinogen*. If, however, whole blood is permitted to clot, the remaining clear liquid is called *serum*. Serum contains no hemoglobin or fibrinogen, because these proteins were removed when the clot was formed. However, the serum contains two other important proteins, viz., *serum albumin* and *serum globulin*. These proteins are not well-defined entities, but they do play an important role in immunochemistry. The liver is thought to be the principal source of regeneration of plasma proteins.

Serum albumins have properties very similar to those of egg albumin. A number of serum globulins have been fractionated, of which α -, β -, and γ -globulins are most important. Serum proteins serve as buffers, regulating the *pH* of the blood. They are also important in maintaining proper water balance between blood and tissues. When food proteins are lacking, serum proteins seem to serve as a protein reserve from which essential amino acids can be drawn for use by body tissues.

The clinical use of blood plasma was especially important in World War II. Blood plasma is used wherever it is necessary to replenish blood proteins. It is of special value in the treatment of burns, shock, and loss of blood.

Some of the characteristics of blood of different types of mammals are given in the table on p. 311.

Blood clotting. When a blood vessel is cut, nature plugs the opening and prevents further loss of blood by forming a clot. Fundamentally, the formation of this clot is caused by the creation of stringy, insoluble *fibrin* from soluble plasma, *fibrinogen*. The stringy, jellylike fibrin enmeshes the corpuscles, forming the clot. However, the process of blood coagulation is a complicated process involving many steps. Under abnormal conditions, blood may clot very slowly, or not at all. In jaundice and certain other liver diseases, blood clotting time may be delayed to the point where bleeding, once started, cannot be stopped unless antihemorrhagic substances, such as vitamin K, are administered. Defective blood clotting can be hereditary. This abnormal

COMPOSITION OF BLOOD

(Results expressed as milligrams per 100 milliliters unless otherwise noted.)

	Man	Albino Rats	Dairy Cattle	Horses	Sheep	Swine	Chickens
Non-protein nitrogen	25-35	45.2	30.1	34	32	35	44
Urea nitrogen	10-15	15.6	12.9	17.8	17.8	19.6	5.6
Uric acid	2-3.5	1.86	2.1	2.45	1.82	2.06	6.39
Creatinine	1-2	1.28	1.4	1.8	1.26	1.42	1.21
Creatine	3-7	6.03	4.3	3.26
Glucose	80-120	122.2	84.1	106	105	97.1	173
CO ₂ capacity of plasma (volume per cent)	55-75	58.9
Chlorides as NaCl	450-500	515.2	49.2	461
Calcium (serum)	9-11	10.0	12.6	9.9
Inorganic phosphorus (serum)	3-4	8-10	4.5	4.0
Hemoglobin (per cent normal haldane scale)	100	92.9
Erythrocytes (per cubic millimeter)	5,500,000	6,500,000	7,900,000	2,800,000
Leucocytes (per cubic millimeter)	5,000-10,000	9,000	5,500	39,000

condition, known as *hemophilia*, does not respond to vitamin K administration.

Prothrombin, according to Seegers and Ware, is a glycoprotein with a molecular weight exceeding that of thrombin. When prothrombin is activated, *thrombin* is formed, in all probability as a degradation product of the prothrombin molecule. A substance known as *thromboplastin* is capable of activating prothrombin to form active thrombin. Substances possessing thromboplastic activity include various tissues, milk, saliva, and snake venoms. The speed with which thrombin is formed from prothrombin depends on the presence of an "accelerator" which Seegers and Ware call *Ac-globulin*. These workers state that *plasma Ac-globulin* possesses no acceleration activity until it is changed to *serum Ac-globulin*. Thrombin seems to be necessary for the formation of serum Ac-globulin from plasma Ac-globulin.

Although much is yet to be learned regarding the chemistry of blood coagulation, it would appear that the following outline gives a fairly accurate picture of the present conception of the probable mechanism of blood clotting:



Thrombosis or intravascular clotting is not uncommon. In such cases a small clot is formed in the circulatory system. If such clots become lodged in a small artery or capillary in the heart or brain, the results may be fatal, unless the clot is dissolved, naturally or by the administration of certain hemorrhagic drugs.

Lymph. In addition to the blood circulatory system there is another system, which is less well defined, known as the *lymphatic system*. This system contains a creamy, colorless liquid called lymph, which resembles blood plasma. The lymph bathes all cells of the body and serves as a medium of transportation for food nutrients from the blood stream to the cells, and waste products from the tissues to the blood stream. Considerable fat is absorbed from the intestinal villi, via the lacteals, and passes by way of the lymphatic system to the blood.

In a sense, lymph might be considered a blood exudate. It has no outstanding chemical characteristics except that the protein content is about half that of blood plasma. The proteins of plasma and lymph seem to be identical so far as general characteristics are concerned. The concentration of some waste products and nutrients seems to be higher than that of blood plasma. This is to be expected, since lymph is intimately associated with tissue cells, with the result that it contains nutrients passing from blood to cells as well as metabolic products passing from cells to blood.

SUPPORTING TISSUES

Bones. Bones form the skeletal framework of the body and are characterized, chemically, by a high proportion of mineral

matter. In the embryo, bones appear first as cartilaginous structures consisting of cells, embedded in a homogeneous intercellular substance which consists largely of proteins. The protein present in the largest amount is called *collagen*. Other proteins, present in less amounts, are *osseomucoid* and *osseoalbuminoid*. When ossification takes place, the intercellular spaces fill with mineral salts of which a salt closely related to tricalcium phosphate predominates. Although authorities are not in complete agreement, evidence indicates that the mineral matter in bone consists of a complex salt, the formula for which may be written $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaX}_2$. The carbonate radical CO_3 usually occupies the X_2 position, but other radicals, such as F_2 , SO_4 , or O , may also constitute this part of the molecule. It is evident that Mg can replace Ca in bone, but the amount of magnesium in bone appears to be relatively constant. Appreciable amounts of lead are found in the bones of animals that have died of lead poisoning. Fluorine is always present in normal bone in small amounts.

Bone marrow, the principal source of red blood cells, consists of organic material containing a protein, called *ossein*, and relatively large amounts of fatty materials.

The following analyses by Ingle give some idea of the gross composition of bone:

COMPOSITION OF BONES FROM MULES

Water	5.34%
Organic matter	37.77%
Ash	56.98%

The organic matter contained:

Fat	7.61%
Nitrogen	3.99%

The ash contained:

CaO	32.28%
P_2O_5	21.86%
SiO_2	0.09%

Zalensky's analyses of bones of humans and of cattle yielded the following data:

	MAN	CATTLE
$\text{Ca}_3(\text{PO}_4)_2$	83.89%	86.09%
$\text{Mg}_3(\text{PO}_4)_2$	1.04%	1.02%
Ca (combined with CO_2 , Cl, F)	7.65%	7.36%
CO_2	5.73%	6.20%

The ossification process is hampered in rickets, osteomalacia, and similar bone diseases. These conditions may be brought about by calcium and phosphorus starvation, by unbalanced Ca:P ratios in the diet, by the absence of sunlight or vitamin D, or by pathological conditions. Lactating animals in high production tend to maintain normal amounts of calcium and phosphorus in the milk, at the expense of their skeletons, unless the diet is adequate in calcium and phosphorus. It is not easy to maintain high producing dairy cattle in calcium balance because of the large demand, by the milk, for this element.

Teeth. These are bony structures consisting of mineral matter embedded in an organic matrix. The principal constituents of teeth are *enamel*, *cement*, and *dentine*. Cement represents the tooth layer covering the root of the tooth. This is a hard material similar to bone, containing about 70 per cent of mineral matter and an organic matrix consisting largely of collagen. The surface of the tooth is composed of an extremely hard material called enamel, which contains from 96 to 98 per cent of mineral matter with a small amount of organic material consisting largely of keratin. The main body of the tooth which lies underneath the enamel and next to the pulp is composed of dentine. This structure contains about 77 per cent of mineral matter and an organic matrix containing collagen.

Enamel, although similar to bone in chemical composition, seems to contain more calcium and less magnesium, sodium, and water. Also, enamel is characterized by the presence of a hydroxyl group, which does not appear to be present in bone. *Dentine* and *cement* occupy an intermediate position between enamel and bone, so far as chemical composition is concerned. Fluorine appears to be an essential constituent which provides a defense against dental caries.

There are two schools of thought regarding the cause and prevention of dental caries. One group emphasizes the impor-

tance of proper diet, involving quantity and quality of calcium and phosphorus, and other dietary essentials such as fluorine and vitamins A, C, and D. The other group believes that dental hygiene plays the major role, that acid-forming bacteria flourish on carbohydrate residues, and that tooth structures are impaired by the action of acid.

Epithelial and connective tissues. These tissues serve to bind and hold the softer body tissues together. They are characterized by great strength, toughness, and elasticity. Epithelial tissues serve as a covering and lining for the body. For example, epithelial tissues serve as lining for the respiratory tract. The outer covering of the body also consists of epithelial tissue and includes such epidermal tissues as skin, nails, hoofs, horn, hair, and feathers. All these tissues are composed of a tough, insoluble albuminoid protein, called *keratin*. The keratins of skin are known as *pseudokeratins*, whereas those in hair and nails are called *eukeratins*.

The most important connective tissues are ligaments, tendons, and cartilages. Tendons and ligaments consist of two types of tissue, viz., white fibrous tissue and yellow elastic tissue. The predominating components of tendons and ligaments are the albuminoid proteins, *collagen* and *elastin*. Collagen is a tough insoluble protein which differs from keratin in that it can be hydrolyzed by digestive enzymes. It contains less sulfur than keratin and forms gelatin when treated with hot water. Commercial gelatin is manufactured from collagen-containing animal tissues.

Tendons contain relatively large amounts of collagen, an albuminoid which contributes strength. Other albuminoids in tendons are *tendomuroid* and *collagen*. Cartilaginous tissues contain elastin, *chondromuroid*, and *chondroalbuminoid*. Chondromuroid is a glycoprotein which yields, on hydrolysis, glucuronic acid, galactosamine, acetic acid, and sulfuric acid. Chondroalbuminoid has physical properties similar to those of keratin and elastin, but it can be hydrolyzed by proteolytic digestive enzymes.

MUSCLE TISSUE

This important tissue constitutes about 42 per cent of the body weight in man. From 50 to 75 per cent of the total body metabolism occurs in this tissue. The highest metabolic rate accompanies strenuous physical exercise, whereas metabolic activity is lowest during complete rest.

Muscle tissues can be classified into three types, depending on their physiological characteristics. Although they differ to some extent in their chemical properties, they have many chemical characteristics in common. The three types of muscles are (1) *voluntary* (striated, skeletal), (2) *involuntary* (smooth, unstriated), and (3) *cardiac*. All muscle tissues contain from 72 to 78 per cent water. The proteins consist of albumins, globulins, nucleoproteins, and albuminoids. In addition, muscles always contain lipids, non-nitrogenous and nitrogenous extractions, inorganic salts, and enzymes.

Muscle proteins. Striated skeletal muscle when subjected to high pressure yields a fluid called *muscle plasma*. On standing, a pinkish colored clot forms, consisting of *muscle hemoglobin* or *myoglobin*. A muscle serum can be separated from the clot. Approximately 40 per cent of muscle tissue consists of a protoplasmic framework called *stroma*, and about 60 per cent is fluid. Muscle is characterized by the presence of four proteins, *two albumins* and *two globulins*. The two globulins, *myosin* and *globulin X*, are present in the greatest quantity. The albumins of muscle are *myogen* and *myoalbumin*. The approximate percentages of myosin, globulin X, myogen, and myoalbumin are of the order of 68, 21, 10, and 1, respectively.

Myosin is the only muscle protein possessing contractile power, and it is also thought to have enzymic properties. It is believed that myosin is unique in that it can act on adenosine triphosphate, thus *releasing energy for muscle contraction*, which the contractile myosin then utilizes. In rigor mortis, the protein myosin becomes irreversibly denatured, coagulates, and becomes insoluble. The softening of muscle, after rigor mortis, is due to autolysis.

Muscle lipids. Muscle lipids consist of *fats*, *phospholipids*, and *cholesterol*. Striated muscle contains the least amount of cholesterol, whereas cardiac muscle is somewhat richer, and smooth (involuntary) muscle contains the largest amount of this sterol. The phospholipid content, on the other hand, is high in voluntary and cardiac muscle but low in involuntary muscle.

Non-nitrogenous extractives. When muscle is extracted with hot water, a number of materials are removed. When traces of proteins are removed by coagulation from the water extract, and lipids are removed with ether, the remaining water-soluble substances are known as "extractives." The non-nitrogenous extractives consist of small amounts of *glucose*, *inositol*, *hexose phosphates*, and *lactates*. Glycogen may be present in appreciable amounts, depending on the activity of the muscle prior to water extraction.

Nitrogenous extractives. Nitrogenous compounds that have been identified in water extracts of muscle include *creatine*, *creatine-phosphate*, *creatinine*, *adenosine triphosphate*, *glutathione*, *purines*, *pyrimidines*, *carnosine*, *anserine*, *choline*, and *acetylcholine*. The physiological significance of some of these important compounds will be discussed in the following chapter.

NERVOUS TISSUE

This type of tissue is without doubt the most important tissue in the human body, for, by its functions, man and the lower animals are clearly differentiated. Nerve tissues are scattered throughout the body. Those that end in the extremities and terminate at the body surface are small in size, but these join others, forming larger trunks, and these eventually join the spinal cord, which in turn is attached to the brain. This type of tissue is highly irritable, receives stimuli, endows us with memory, and controls our psychic and motor activities.

A study of the chemistry of brain and nervous tissue shows that these tissues differ materially from other tissues. For example, they contain relatively large amounts of alcohol-ether-soluble materials (lipids). Although the lipid content of nervous

tissues is high, it is interesting to note that ordinary fats are not present. The lipids that predominate are *cholesterol*, *phospholipids*, and *glycolipids*. Three types of phospholipids are found in nervous tissue. These are *lecithins*, *cephalins*, and *sphingomyelins*, which differ primarily in the type of nitrogenous base combined in the phospholipid molecule. Lecithin contains choline, the cephalins contain *hydroxyethylamine*, and the characteristic bases in the sphingomyelins are *choline* and an amino alcohol, *sphingosinol*.

The *glycolipids* are sometimes called *cerebrosides*. Two members of this group are *phrenosin* and *kerasin*. These are glucoside-like compounds which yield galactose in addition to fatty acids and sphingosinol. Cholesterol is a sterol which was discussed in a previous chapter. The water content of nervous tissue is relatively high, but it tends to become less high as the organism matures. About one-half the dry matter of the human brain consists of proteins such as *albumin*, *globulin*, *collagen*, *nucleoprotein*, and *neurokeratin*.

Extractives that have been isolated from brain tissue include *creatine*, *lactic acid*, *purines*, and *inositol*. Inorganic salts are also normal constituents of nervous tissue. Of these the alkaline phosphates are of particular importance.

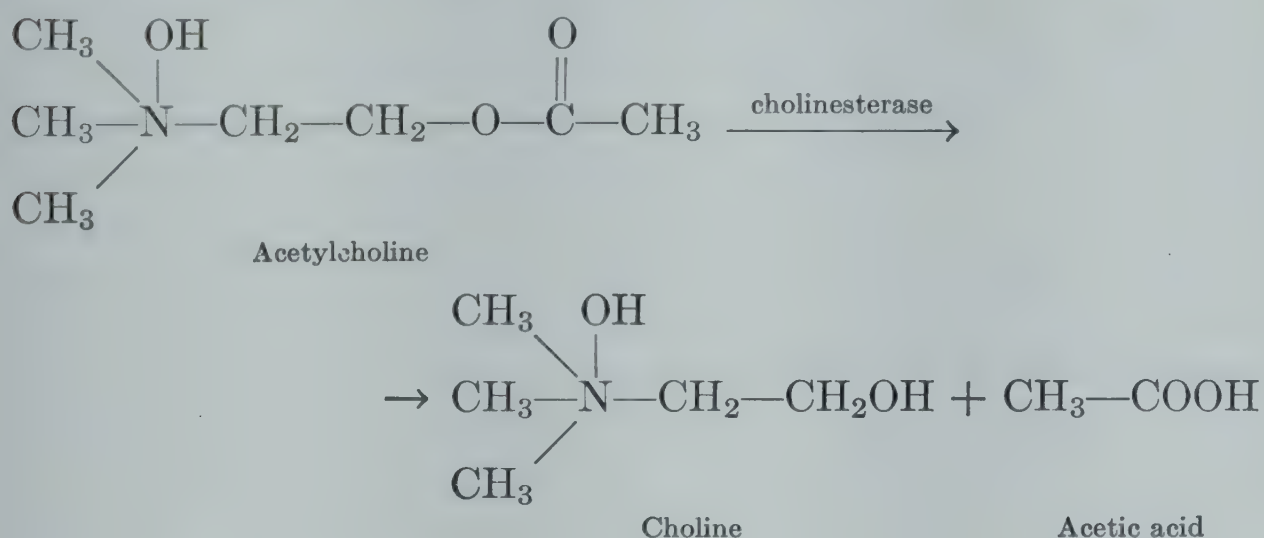
The energy metabolism of brain tissue is abnormally high, as measured by oxygen consumption. No glycogen is found in brain tissue, with the result that the brain must depend on blood glucose as its chief energy-producing substance. Thus it is fortunate that nature has endowed the brain with a generous blood supply.

When blood sugar is too low, as when overdoses of insulin are administered, patients become dizzy and confused and often become unconscious. This principle has been applied with some success in treating certain types of mental cases (dementia praecox). Patients are treated by what is known as "insulin shock therapy," which often brings about improvement of the mental condition, following recovery from the insulin shock.

Although *acetylcholine* is not present in nervous tissue in appreciable amounts, it should be mentioned at this point. This important compound is formed by the union of choline and acetic acid. This substance is thought by some workers to be respon-

sible for the transmission of nerve impulses from nerves to muscles or from nerve to nerve across synapses. In all probability the transmission of nerve impulses involves changes which are chemical and electrical in nature.

The synthesis and hydrolysis of acetylcholine is thought to be controlled by the enzyme *cholinesterase*.



RESERVE TISSUES

Most of the tissues described in previous pages have had definite duties to perform. In other words, these tissues constitute what Armsby calls "the working machinery" of the body. Another function of certain cells is the storing of reserve food supplies that may be called upon in time of stress.

Fatty tissues. Practically every tissue in the animal body contains fat in some form, although certain tissues are characterized as fatty tissues. Adipose tissue is a representative fatty tissue and is usually laid down in the abdominal region in humans. The nature of adipose tissue, kidney fat, and intestinal fat is very largely dependent upon the kinds and amounts of fatty acids in combination. If stearic and palmitic acids predominate, the tissue is firm and hard, whereas the reverse is true if oleic acid is present in sufficient amounts. Body fats contain stearic, palmitic, and oleic acids, although other fatty acids are present. Fatty tissues in hogs usually contain larger percentages of fat and lower percentages of water than those of sheep or cattle. Hog tissue may contain as much as 92 per cent fat. As a rule the subcutaneous fats (those immediately

under the skin) are softer and contain more oleic acid than the deeper fats. Although animal fats do not appear to differ appreciably from plant fats in chemical composition, they possess different nutritive properties, owing to the difference in concentration of fat-soluble vitamins. Beef and mutton tallow, representative of the hard fats, are characterized by large amounts of stearic and palmitic acids, whereas beef oil is rich in oleic acid. Human fat falls between these extremes.

GLANDULAR TISSUES

Certain tissues in the body are capable of elaborating and discharging secretions of the utmost importance to the welfare of the animal body. These are known as glandular organs, and they possess specialized cells known as *gland cells*. The glandular tissues do not, of themselves, differ strikingly in chemical composition from other body tissues, but they are of interest because of the chemical substances they elaborate.

These tissues may be classified in two groups: (1) glands of external secretion, with ducts which secrete their chemical materials in such a manner that they may pass from the body, and (2) glands that possess no ducts and secrete their chemical materials directly into the blood stream where they are carried to other tissues. The latter are known as *ductless glands*, *endocrine glands*, or *glands of internal secretion*.

Glands of external secretion

This group of glands is of great importance to the body since the secretions are used for the digestion of food, the feeding of the young, or the excretion of waste products. As we have said, these secretions are eliminated from the body eventually, unless some of their products are absorbed in the digestive tract. The following list of glands and their secretions will give the reader some idea of this type of tissue and its function:

1. Salivary glands, secreting saliva. See salivary digestion.
2. Gastric and intestinal glands, secreting digestive juices. See gastric and intestinal digestion.

3. Mammary glands, secreting milk.
4. Pancreas and liver glands secreting pancreatic juice and bile, respectively. See intestinal digestion.
5. Sweat glands, secreting perspiration.

Glands of internal secretion

Ductless glands are known in medicine as the "endocrine glands" or "endocrine organs," and the division of medicine dealing with the study of these glands is known as "endocrinology." These tissues are unique in that they manufacture and secrete into the blood stream chemical substances necessary for the normal functioning of body tissues. Apparently these chemical substances are necessary to stimulate other organs to function in a normal manner. These body regulators, manufactured by the ductless glands, are known as *hormones*, a term meaning "chemical messengers." A few of these glands and their hormones will be discussed. It might be well to state that extracts from some of these glands have been studied from which as yet no definite hormones of known chemical structure have been isolated. A number of compounds have been isolated from some of the endocrine organs, however, and their chemical structure has been determined. In fact it is no longer necessary to depend on animal organs for many of these hormones, for they are now being synthesized by the manufacturing organic chemist.

Gastrointestinal glands. The hormones produced in the digestive tract have been mentioned in our discussion of digestion. *Gastrin* is a hormone produced by cells in the pyloric mucosa. It is carried in the blood to cells in the mucosa of the fundic portion of the stomach, where it stimulates the flow of gastric juice.

When the HCl of the stomach contents reaches the small intestine, cells in the intestinal mucosa are stimulated to produce the hormone, *secretin*. This hormone is carried by the blood to the pancreas, where it stimulates the flow of pancreatic juice. Simultaneously a second hormone from the intestinal mucosa, *pancreozymin*, stimulates the production of pancreatic enzymes. A third hormone, originating in the intestinal mucosa, is called *enterogastrone*. The production of this hormone is thought to

be stimulated by the presence of fatty foods in the intestine. This hormone is thought to slow up gastric digestion in order that fat digestion in the intestine may be accomplished more efficiently.

Pancreas. This organ functions not only as a source of pancreatic juice but also as an endocrine tissue. When this gland is removed, animals develop diabetes, a metabolic disorder in which the body loses its power to oxidize sugars and fats. In this disease the blood sugar becomes abnormally high (*hyperglycemia*) and unburned sugar spills over into the urine. The latter condition is called *glycosuria*. The pancreatic hormone, which prevents diabetes, has been isolated in crystalline form and is known as *insulin*.

Insulin is secreted by special areas of cells in the pancreas, known as the *islands of Langerhans*. When these cells become diseased and fail to function, insulin is not secreted and diabetes results.

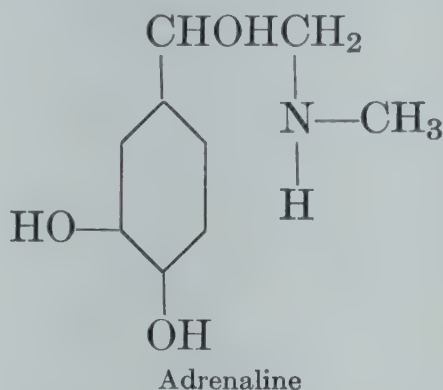
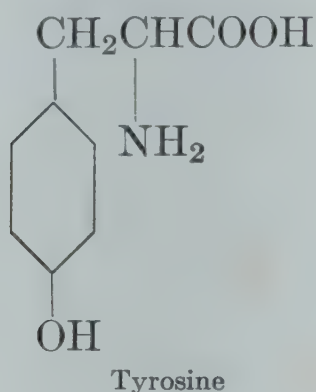
Insulin is a protein of unknown chemical structure. Since it cannot be synthesized, medicine must depend upon natural insulin prepared commercially from pancreatic glands. It cannot be administered orally because it is destroyed by proteolytic enzymes. Consequently it is administered more efficiently by injecting it in the form of the water-soluble hydrochloride. One-eighth milligram of standardized insulin hydrochloride contains one unit of insulin. Injected insulin lowers the blood sugar very rapidly and often causes insulin shock. Furthermore the insulin effect disappears rather quickly. To overcome this insulin has been combined with zinc and protamine, forming a complex which is effective over a greater period of time, decreases the possibility of insulin shock, and reduces the number of daily injections.

Insulin functions in stimulating the normal oxidation of sugar and the normal deposition of glycogen in liver and muscle. Failing to oxidize the sugars, the body attempts to obtain its energy by the oxidation of fats and ketogenic amino acids. As a result, acetone bodies accumulate, causing *acidosis*.

Adrenal glands. The *adrenal glands*, sometimes called the *suprarenal glands*, are small glands situated on the upper end of each kidney. In human beings, each gland weighs about

3 grams. Each adrenal gland consists of an inner portion, the *medulla*, and an outer portion, the *cortex*. Each anatomical portion has its own endocrine characteristics.

Adrenal medulla. The hormone secreted by the medulla is known as *adrenaline* or *epinephrine*. In chemical structure it is closely related to the amino acid, tyrosine.



When adrenaline is injected, it causes constriction of the arterioles, increases blood pressure, and stimulates the heart. It is useful in surgery in reducing hemorrhages. In anger or fear the amount of adrenaline is automatically increased, followed by a marked increase in blood sugar. Cannon has suggested that this is nature's method of providing ready fuel for emergencies.

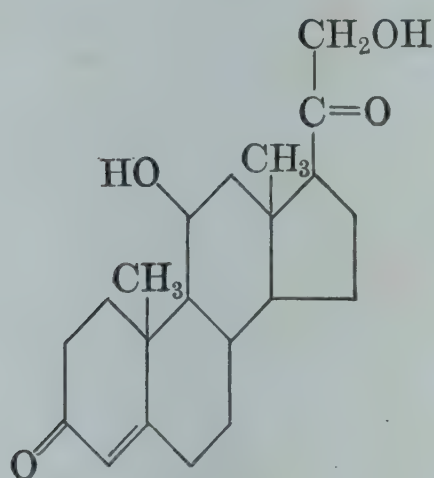
A solution consisting of one part of adrenaline hydrochloride to 300,000,000 parts of water will cause physiological response in animals. Dr. Haskins of Harvard University has calculated that, in order to obtain the above-mentioned dilution, 40 miles of water carts (220 carts to the mile and 625 gallons to the cart) would be required for 1 ounce of adrenaline. In spite of the physiological effects just described, there is no evidence that the adrenal medulla is essential for life. The medulla can be removed without apparent harm to experimental animals.

Adrenal cortex. Although the medulla does not seem to be essential for life, animals will die if the adrenal glands are removed. This is because the adrenal cortex plays a very important physiological role. When the cortex is destroyed by disease, humans become ill and eventually die. This is known as Addison's disease. This disease is characterized by loss of sodium, increase of blood potassium, emaciation, low blood pressure, hypoglycemia, and abnormal pigmentation of the skin.

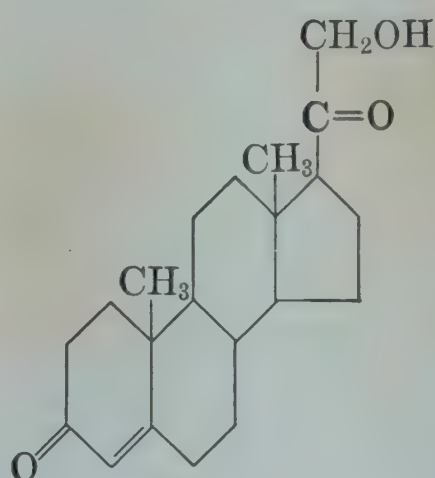
Cortical extracts usually prevent and cure this disease. The curative effect of cortical extract is due to the presence of cortical hormones.

About 30 steroids have been isolated from the adrenal cortex. The chemical configurations of these have been determined, and several of them have been synthesized. A hormone called *corticosterone* is one of the most active of the cortical hormones. The others are derivatives of corticosterone. *Desoxycorticosterone*, one important derivative of corticosterone, has one less oxygen and has found wide use in medicine.

As these words are written, workers at the Mayo Clinic have announced that "compound E," an adrenal cortical hormone, offers great promise in the treatment of arthritis and rheumatic fever. Compound E has been identified as 17-hydroxy-11-dehydrocorticosterone. At present this compound is prepared by "partial synthesis," by modifying the chemical structure of cholic acid obtained from bile. It is hoped that a starting material more abundant than bile can be found in order that the new compound will be available to clinicians for treatment of arthritis and rheumatic fever at a price the patient can afford. The amount now available is insufficient for experimental purposes, and the cost of production is exorbitant.



Corticosterone



Desoxycorticosterone

None of the individual steroids will cure Addison's disease, although the disease will respond to cortical extract, indicating that other (unidentified) factors are involved. A group of *adrenogenital hormones* are produced in the cortex. These have to do with the accentuation of sex characteristics in males and

females. These are similar to, but not identical with, hormones produced by the testes and ovaries.

Thyroid gland. This gland consists of two lobes connected by an isthmus and, in man, is attached to the trachea at that point of the neck or throat which is adjacent to the suprasternal notch. When this gland enlarges, the disease is commonly called *goiter*. "Big neck" or simple goiter is associated with lack of iodine in food and water supplies. Normal human thyroids contain from 12 to 25 milligrams of iodine, whereas glands of persons afflicted with goiter may contain little or none. Goiter is not peculiar to man but occurs in cattle, horses, sheep, and swine. It has even been produced experimentally in fresh-water fish.

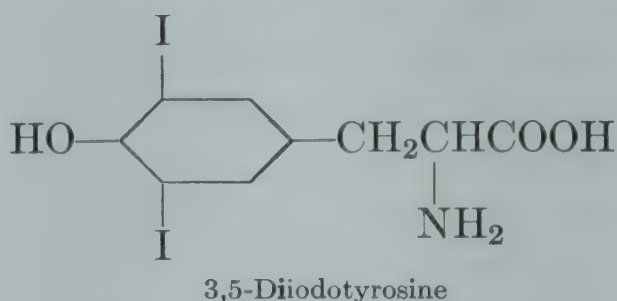
Hypothyroidism. If the thyroid gland does not develop in early life, children do not grow. They become dwarfed and are known as *cretins*, and the condition is known as *cretinism*. These abnormal dwarfs have low mentality, bowed legs, coarse hair, and thick skin. In adults hypothyroidism is called *myxedema*. These cases usually respond dramatically to the administration of desiccated thyroid glands or thyroid hormone. Hypothyroid cases exhibit abnormally low metabolic rates.

Hyperthyroidism. As this name indicates, the thyroid gland becomes abnormally active, causing a disease known as *exophthalmic* or *toxic goiter*. A typical symptom is pronounced bulging of the eyes. In this disease the metabolic rate is higher than normal, patients are afflicted with insomnia and are nervous and often irritable. From a metabolic standpoint victims of hyperthyroidism burn body tissues more rapidly than nature intended, lose weight, and may succumb to cardiac failure unless a portion of the excessively active gland is removed.

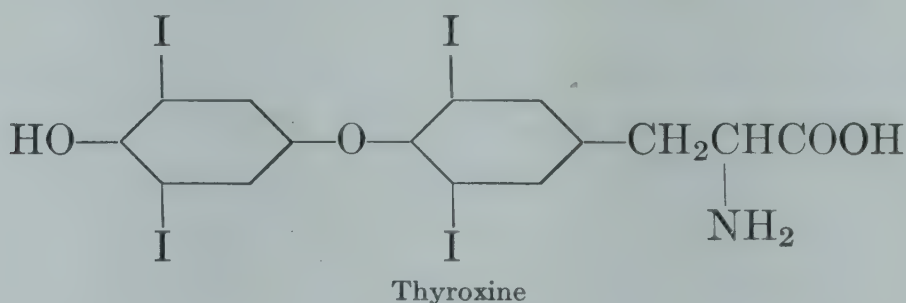
One compound produced by the thyroid gland is called *thyroxine*. This compound contains iodine. The normal thyroid gland accounts for about one-third of the total iodine of the body. It is for the purpose of supplying the normal iodine requirement of humans that many municipalities add iodine salts to their water supplies. Iodized table salt also plays an important dietary role in furnishing extra iodine.

Thyroxine is closely related to the amino acid, tyrosine. Another compound, diiodotyrosine, is believed to be the precursor

of thyroxine, since the former is also a normal constituent of the thyroid gland.



Thyroxine contains four atoms of iodine and is known, chemically, as 3,5,3'5'-tetraiodothyronine, or β -[3,5-diiodo-4(3',5'-diiodo-4'-hydroxyphenoxy) phenyl]- α -aminopropionic acid.



A *thyroglobulin* can be isolated from the thyroid gland. Recent work indicates that thyroglobulin may be the principal thyroid hormone, although it probably owes its activity to the presence of thyroxine, which is a part of the protein molecule.

A so-called "goiter belt" extends in the United States from Montana to and including the states bordering on the Great Lakes. In these regions the water, soil, and vegetation are relatively low in iodine. The iodine content of water in Lake Superior is so low that it has been estimated that a person would have to drink Lake Superior water for 2000 years to obtain enough iodine to supply the normal iodine requirement of the body.

Parathyroid glands. These glands are so closely associated with the thyroid gland that very skillful surgery is required to separate them. In humans there are at least four types of parathyroid tissues or glands. Two of these, the *internal parathyroids*, are embedded in the thyroid glands, and two others, the *external parathyroids*, lie behind the thyroid gland.

When the parathyroids are removed, experimental animals die within two weeks with many typical symptoms, the most distinctive of which is an increase in blood phosphorus and a

lowering of blood calcium, accompanied by characteristic muscle contractions (tetany).

The administration of parathyroid hormone (parathormone) will relieve tetany, increase blood calcium, decrease blood phosphorus, and increase the phosphatase activity of blood serum. Occasionally a type of tumor develops on the parathyroid glands causing hyperparathyroid activity. This is known as von Recklinghausen's disease. In this disease, bones become decalcified and blood calcium values are abnormally high.

Pituitary gland. This small gland, also called the *hypophysis*, is situated at the base of the brain. Anatomically it consists of three structures, the *anterior lobe*, the *intermediate lobe* or *pars intermedia*, and the *posterior lobe*. The anterior lobe is the largest and most important portion of the gland, although the posterior lobe is also important from the standpoint of endocrine activity. Not much is known regarding the hormone-producing characteristics of the *pars intermedia*.

So many important physiological functions have been attributed to the pituitary gland that it is difficult to record a satisfactory summary of its more important functions. If the pituitary is removed, a series of abnormalities become evident. Young mammals cease to grow, and physical and mental activities are retarded. If the gland is removed from the mature mammal, other endocrine glands tend to atrophy and diminish in hormone production. This is true for the thyroid, the parathyroid, the adrenals, the testes, and the ovaries. Appetite decreases, followed by loss of weight, and protein, fat, and carbohydrate metabolism becomes abnormal. In other words, this "master gland" seems to control the activity of most of the other endocrine organs.

Anterior lobe. *Reduced activity* of the anterior lobe of the pituitary gland leads to *dwarfism* in young animals, accompanied by retention of infantile characteristics and lack of sexual development. Administration of extracts of the anterior lobe prevent and alleviate these symptoms. *Hyperactivity* of the growth hormone in the anterior lobe causes *gigantism* in which young mammals tend to grow to gigantic proportions. Adult humans tend to develop bone malformations, particularly in the bones of the face. This is known in medical language as *acromegaly*. To date, no specific hormone has been isolated

to which these abnormalities can be ascribed. It is evident, however, that the anterior lobe produces a number of hormones known as tropic hormones. For example, the existence of a *thyrotropic hormone* is postulated, the function of which is to stimulate the production of thyroxine by the thyroid gland. The paratropic hormone regulates the proliferation of cells in the parathyroid gland. Similarly, other tropic hormones are postulated. These may be summarized as follows: *adrenotropic hormone* (affecting the adrenals), *prolactin*, the *lactogenic hormone* (stimulating the mammary gland), *gonadotropic hormones* (stimulating production of sex hormones), *diabetogenic hormones* (regulating blood sugars), and *insulinotropic hormone* (controlling insulin production and action).

Posterior lobe. The hormones of the posterior lobe of the pituitary gland have not been clearly defined. It is known that posterior lobe extracts contain a substance which causes contraction of the smooth muscles of the uterus. Commercial preparations have been used with success in obstetrics to stimulate uterine contractions. Kamm has isolated a fraction which he calls α -*hypophamine*. This material is also known as *oxytocin* and *pitocin*.

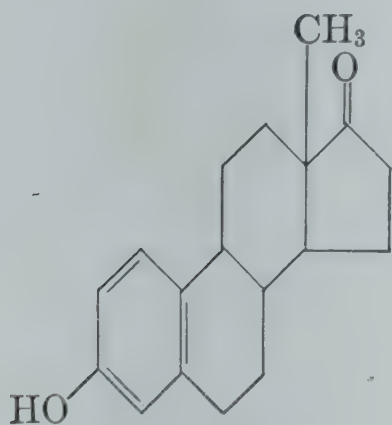
Another fraction of the posterior lobe extract isolated by Kamm is called β -*hypophamine*, *vasopressin*, and *pitressin*. This preparation causes a rise in blood pressure (pressor activity) and decreases excessive flow of urine (antidiuretic effect). The possibility exists that all these effects are brought about by a single hormone.

Placenta. Aschheim and Zondek discovered a gonadotropic hormone, known as *chorionic gonadotropin*. This hormone is produced by the placenta and is excreted into the urine soon after pregnancy occurs. When pregnancy urine is injected into immature female mice, it causes hemorrhage of an ovarian follicle within a period of four days. This has become the basis of a pregnancy test which has achieved real success in human medicine. The Friedman pregnancy test is based on the same principle. Human pregnancy urine is injected into mature unmated female rabbits. If the placental hormone is present in the urine, follicular rupture and corpus luteum formation occur within one or two days.

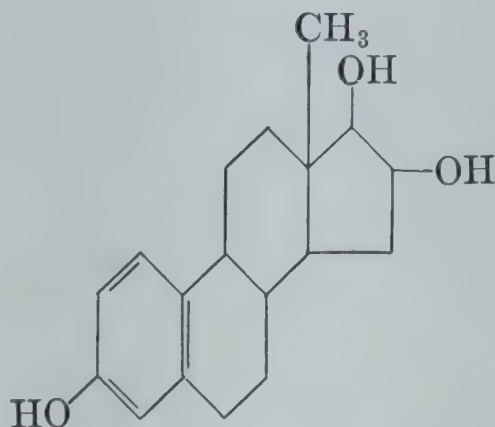
Reproductive glands. The organs of reproduction responsible for the production of sex hormones are the ovaries and the testes.

Ovaries. The cyclic phenomenon of menstruation in the human is stimulated by a hormone called *estrone*. This hormone is produced in the growing ovarian follicle. The growth of the follicle, in turn, is stimulated by a gonadotropic hormone produced by the anterior lobe of the pituitary gland. The rupture of the follicle liberates the ovum which passes to the uterus by way of the Fallopian tube. The ruptured follicle then develops into a yellow body called *corpus luteum*, which, in turn, produces a hormone called *progesterone*. If the ovum is not fertilized, the corpus luteum grows for a few days and then gradually disappears to be replaced at monthly intervals by new follicles and ova.

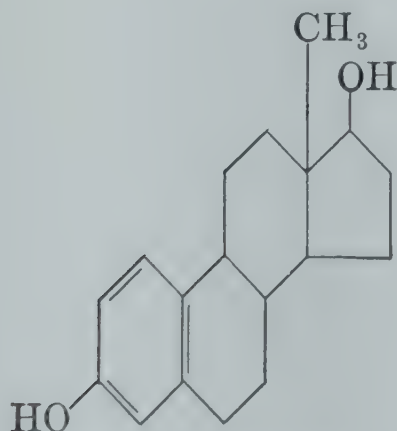
Modern organic chemistry has identified a number of estrogenic compounds, among which are (1) *estrone* or *theelin*, (2) *estriol* or *theelol*, (3) *estradiol* or *dihydrotheelin*, (4) *equilin*, and (5) *equilenin*. These estrogens are steroids.



Estrone

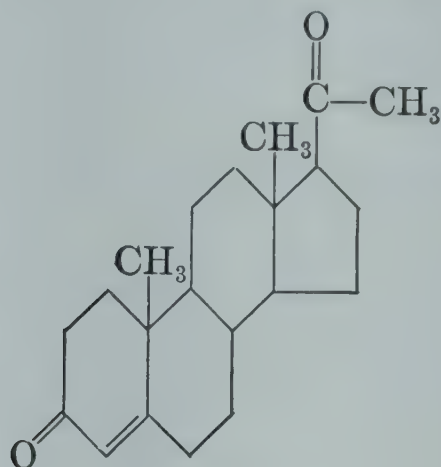


Estriol



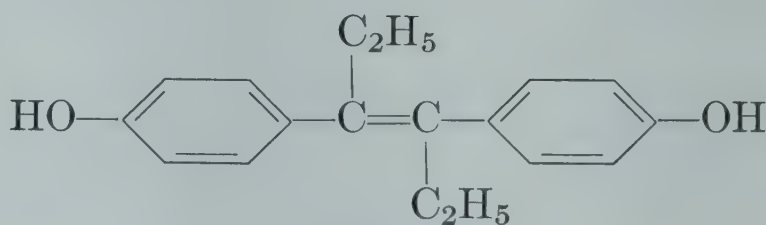
Estradiol

Some investigators believe that the more potent estradiol is the true ovarian hormone which is converted to estrone, and that estriol is formed from estrone. The body seems to use glucuronic acid to detoxicate estriol, forming physiologically inactive estriol glucuronate, which is excreted. The corpus luteum hormone, *progesterone*, continues uterine changes which were initiated by the estrogenic hormones, estrone or estradiol. Implantation of the fetus on the wall of the uterus does not occur if progesterone production is lacking.



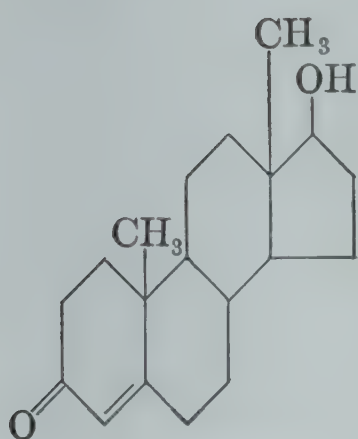
Progesterone

Stilbestrol. This is a synthetic estrogen derived from stilbene ($\text{C}_6\text{H}_5 \cdot \text{CH}:\text{CH} \cdot \text{C}_6\text{H}_5$). It is not chemically related to the natural estrogens, but the diethyl derivative is about three times as potent as estrone. This compound has found wide use in modern medical practice.

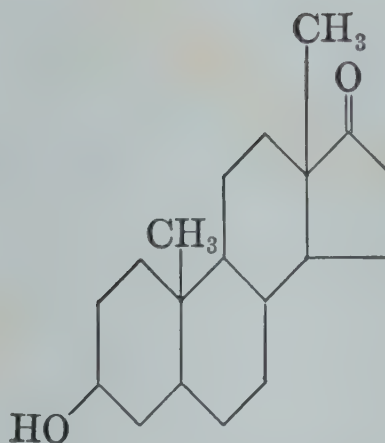


Diethylstilbestrol

Testicular hormones. Hormones produced by testicular tissues are known as *androgens*. They are also present in urine. Although they are termed "male sex hormones," they have estrogenic properties. So far as the male is concerned, these hormones serve to bring about normal development of the male reproductive organs and to preserve and maintain secondary male characteristics. For these reasons they are called *androgens*. Two typical androgens are *testosterone* and *androsterone*.



Testosterone



Androsterone

Thus it can be seen that the “male” and “female” steroids are closely related chemically. It is hoped that this very brief and incomplete discussion of the chemistry of animal tissues will be useful in helping the student obtain a better understanding and appreciation of the chemical changes that occur during metabolism.

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18 • The Vitamins

INTRODUCTION

Hippocrates (460–370 B.C.) is said to be the first to attempt to explain the vitalizing function of food in animal nutrition. It was his conception that good foods contain a single mysterious chemical substance necessary for life, health, and growth. If a food or feed failed to promote health and growth, it was supposed to be lacking in this vital substance.

It is interesting to note that this conception regarding the essentiality of a single chemical substance for good nutrition persisted for hundreds of years. As late as 1834 the eminent scientists, Dr. William Beaumont in America and Dr. William Prout in England, were still writing and philosophizing about a single chemical substance in foods which was necessary for growth and life. This essential nutrient was called “aliment” by these pioneer workers.

During this period a French physiologist, François Magendie (1783–1855), advanced the hypothesis that the nitrogen-containing tissues of the animal body are derived from the nitrogenous constituents of foods. With the aid of the German agricultural chemist, Justus von Liebig, evidence accumulated to show that the animal body requires at least three types of chemical substances for normal life processes. Today we recognize these substances as proteins, fats, and carbohydrates. Nutrition scientists finally concluded that, although proteins are essential for tissue building, carbohydrates and fats function almost entirely as sources of heat and energy.

With the development of analytical chemistry it became possible to separate and identify many other essential food constituents, including several mineral elements. Gradually the

conviction grew that the nutritional requirements of the growing animal are much more complex than had been thought. In spite of this growing conviction nutrition workers were forced to evaluate foods on the basis of their content of protein, fat, carbohydrate, crude fiber, and ash, with the emphasis on protein content and energy value.

As early as 1881 Lunin had devised so-called "purified rations," consisting essentially of purified proteins, carbohydrates, fats, and inorganic salts, which were fed to growing mice. The mice did not grow, and died, unless a small amount of milk was added to this presumably complete diet. Lunin concluded that milk contains an essential substance (or substances) other than protein, fat, carbohydrate, and mineral salts which is necessary for life and growth. In all probability these were among the first "biological response" studies in which small animals and artificial rations were employed. In 1906, Hopkins of England conducted similar experiments, using more highly purified diets. He also concluded that milk contains essential nutrients, other than proteins, fats, carbohydrates, and mineral salts, and he suggested that these unknown essential constituents be named "accessory food factors."

In 1897 Eijkman, a Dutch physician, produced experimental beriberi in chickens by feeding white (polished) rice. The disease could be prevented and cured by feeding whole (red) rice or alcoholic extracts prepared from rice bran. These experiments were confirmed in 1911 by Funk, a Polish chemist. Funk finally succeeded in isolating a crystalline substance from rice bran extract which possessed curative properties identical with those possessed by the rice bran extract. The curative crystalline substance contained hydrogen, carbon, oxygen, and nitrogen and possessed basic properties similar to those characterizing the *amines*. Since the substance was *vital* for the health of his experimental birds, he suggested that such substances be called "vitamines." In spite of the objections of some scientific workers, the name *vitamine* appealed to the majority of nutrition workers, with the result that *vitamine* soon became an official term in nutritional and medical literature. Subsequently the final *e* was dropped in order that vitamins might not be confused with the plant alkaloids, such as morphine and strychnine, the names

of which end in "ine." As a result the officially recognized term is now vitamin, in which the first *i* is long, the second *i* is short, and the accent is on the first syllable.

As research work progressed, nutrition chemists added additional evidence to show that many types of nutrients are necessary for growth and reproduction. Osborne and Mendel of Yale University and McCollum of the University of Wisconsin proved that growing rats require a fat-soluble factor for normal growth. This factor, found in butter fat and certain liver oils, became known as vitamin A. Funk's anti-beriberi factor was found to be an essential nutrient for growth and was named vitamin B. When it was finally established that scurvy can be prevented and cured by a chemical substance present in citrus and other fruits, the scurvy-curing factor became known as vitamin C. Similarly a fat-soluble substance in fish-liver oils, which prevents the development of rickets, was designated as vitamin D, and the term vitamin E was given to a fat-soluble factor in wheat-germ oil, which prevents a type of sterility in rats.

Thus it became clear that a new nutritional concept had developed in which it was no longer possible to explain perfect nutrition on the basis of a few essential substances. In fact, research workers have continued to add an increasing number of chemical substances to the long list of essential nutrients required by living organisms. During recent years the chemist has been able to prove that the old vitamin B is really a "complex" consisting of many water-soluble vitamins. Likewise, each of the fat-soluble vitamins (A, D, and E) is now known to be multiple in nature.

Many vitamins have been isolated in pure crystalline form, their chemical configuration has been established, and several vitamins have been synthesized on a commercial scale. In fact the amount of money spent for commercial vitamin preparations in the United States is said to be in excess of \$100,000,000, annually.

It is not possible to state with accuracy the number of vitamins and vitaminlike substances that are supposed to be necessary for normal health and well-being. At least forty vitamins have been described in the literature. In subsequent discussions we



FIG. 15. A typical case of thiamine deficiency (polyneuritis or avian beriberi) in the pigeon. The head is retracted and the bird loses control of muscles in wings and legs.

shall confine our attention to those water-soluble and fat-soluble factors which are usually recognized by scientific workers and of which the chemical and physiological characteristics have become quite firmly established.

For convenience of discussion, vitamins can be classified according to their solubility. Those characterized by solubility in water are known as "water-soluble vitamins," whereas those soluble in fat solvents are called "fat-soluble vitamins."

WATER-SOLUBLE VITAMINS

Eijkman, Grijns, and Funk were among the first to study water-soluble B, the factor which prevents and cures the oriental disease known as beriberi. Eventually it was discovered that water-soluble B is a complex consisting of several water-soluble vitamin fractions. One of these fractions was finally isolated in pure crystalline form and was found to be the vitamin which is most specific in preventing and curing polyneuritis (beriberi). Eventually this vitamin became known as vitamin B₁ or the "antineuritic vitamin." Because vitamin B₁ is specific in alleviating nervous symptoms of polyneuritis, the vitamin is said to possess antineuritic properties. As a result some European workers refer to vitamin B₁ as "aneurin."

Finally, when the chemical structure of vitamin B₁ became

known, it was given the chemical name *thiamine*, which is now recognized as the preferred official designation of this vitamin. Alphabetical designations of vitamins are being abandoned as rapidly as suitable chemical names can be established.

Thiamine (B₁)

Thiamine deficiency. Thiamine deficiency is known clinically as beriberi. This disease has been confined largely to the rice-eating countries of the Far East (China, Japan, India, and the Philippine Islands). Whole rice contains relatively adequate amounts of this vitamin which is concentrated in the silver skin or polish removed with the bran when rice is polished.

Since white (polished) rice is the principal food eaten by the low-income classes in the Orient, it is not surprising that thiamine deficiency should result. Beriberi was quite common among prisoners in Japanese prison camps during World War II. People afflicted with beriberi or animals afflicted with experimental thiamine deficiency display similar clinical symptoms. The first noticeable symptom is loss of appetite (anorexia). This is followed by loss of body weight, intestinal sluggishness (stasis), lowered rate of heart beat (bradycardia), and development of nervous symptoms. Muscular atrophy is followed by paralysis, and post-mortem examinations reveal enlargement of the heart and degeneration of the nervous system. Heart failure and insanity are characteristic results of beriberi.

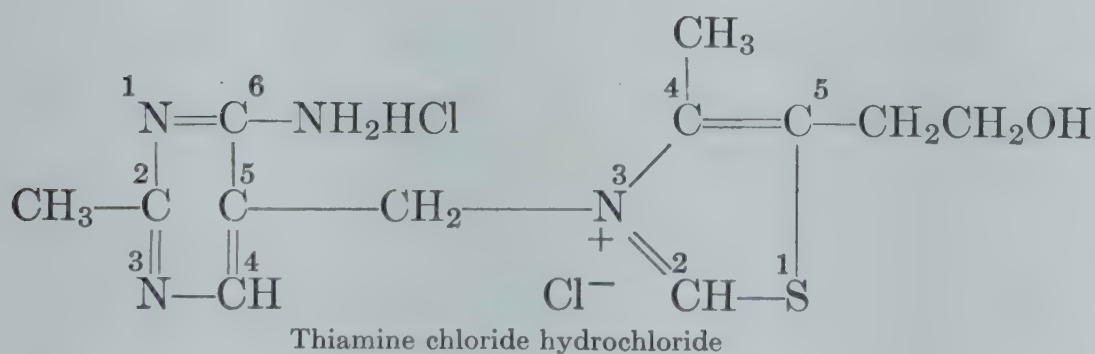
The term "polyneuritis" is applied to thiamine deficiency because the peripheral nerves are invariably affected. It must be borne in mind, however, that people afflicted with beriberi usually subsist on diets which are also deficient in other essential food factors, such as proteins, mineral salts, and other vitamins. Consequently it is almost impossible to find cases of uncomplicated thiamine deficiency in humans. Invariably such patients are really suffering from multiple deficiencies of which thiamine deficiency is but one.

Chemistry. From 1906 to 1926 chemists were busy devising ways and means of concentrating and isolating the antineuritic factor. Early workers knew that the curative factor was soluble in water and dilute alcohol, that it was thermolabile, and that it was diffusible through membranes. It could be adsorbed on

fuller's earth and charcoal and (by this means) separated from many water-soluble extraneous materials. It could also be precipitated from solutions with phosphotungstic acid. During the period 1926 to 1934 workers in Batavia, Japan, Germany, England, and the United States described the chemical and physical properties of a crystalline substance, isolated from rice bran and yeast, which possessed antineuritic properties of marked potency.

By 1934 R. R. Williams and co-workers in the United States had succeeded in developing methods that yielded approximately 5 grams of the crystalline antineuritic vitamin from 1 ton of rice polishings. Although this amount appears to be extremely small to the layman, it was sufficient for quite comprehensive research work. As a result, Williams and co-workers were able to study the degradation products produced by splitting the molecule with sodium sulfite.

One cleavage product was identified as a pyrimidine type of compound, and the other cleavage product was a basic quaternary compound containing sulfur (thiazole). As soon as these degradation products were identified it was possible to postulate the probable chemical configuration of the original vitamin molecule. Final proof for the structure was obtained when the molecule was synthesized in the laboratory. The synthetic product was found to be identical, chemically and biologically, with the crystalline product obtained from rice polishings. The American workers named the vitamin "thiamine," to indicate that it contains basic nitrogen and sulfur. The chemical name of thiamine is 4-methyl-5- β -hydroxyethyl-N{[2-methyl-6-aminopyrimidyl-(5)]-methyl}thiazolium chloride hydrochloride. It will be noted that the pyrimidine nucleus is connected to the thiazole ring by a methylene bridge and that the thiazole ring contains a quaternary nitrogen which is very active chemically.



Properties. Thiamine is relatively stable to dry heat, but it is readily destroyed when heated in the presence of water. The presence of acids will retard thiamine destruction, whereas destruction is hastened in alkaline media.

Thiamine is easily oxidized in the presence of mild oxidizing agents to form a fluorescent pigment called *thiochrome*. This chemical characteristic is used as the basis for a method for the quantitative determination of thiamine. Because of the presence of the hydroxyl radical, thiamine is capable of forming esters. The pyrophosphoric acid ester of thiamine is a coenzyme called *coccarboxylase*, which is the principal form in which thiamine exists in body tissues.

Function. This coenzyme, thiamine pyrophosphate (coccarboxylase), functions as part of the enzyme, carboxylase, which catalyzes the decarboxylation and carboxylation of pyruvic acid, an intermediate in carbohydrate metabolism. When free thiamine is ingested it must be phosphorylated in the body tissues. This is accomplished by accepting phosphoric acid from adenosine triphosphoric acid. Although enzymes are synthesized in the animal body, it would appear that coenzymes (or their precursors) must be made from dietary ingredients.

In beriberi, owing to lack of thiamine, pyruvic acid is not oxidized and accumulates in the blood and tissues. When thiamine is fed, the patient improves or recovers and the accumulated pyruvates decrease. This is explained on the basis that the body carboxylase cannot complete the oxidative process until the coenzyme, coccarboxylase, is supplied. Coccarboxylase cannot be present in adequate amounts if the dietary thiamine intake is not adequate.

The nervous system is directly affected in thiamine deficiency. This specific effect is attributed to the fact that the cells of nervous tissues utilize only carbohydrate for energy purposes. It must be remembered, however, that thiamine is essential for normal metabolism of all body tissues. Evidence also points to the fact that fat has a sparing action on the thiamine requirement. This is explained on the basis that thiamine is not essential for fat oxidation. Deficiency symptoms are enhanced and the thiamine requirement is increased when the carbohydrate intake is increased and the fat intake is decreased.

International thiamine standard. In order that all laboratories over the world shall be able to express the thiamine content of foods in the same terms, it has been necessary to establish an international standard of measurement. This standard is pure crystalline thiamine chloride hydrochloride. As a result research workers are free to use biological, microbiological, or chemical methods in measuring vitamin content of foods so long as they use pure crystalline thiamine as the reference standard of measurement.

Thiamine requirements. Minimum requirements of human beings and domestic animals for thiamine cannot be stated with any degree of accuracy because the requirement depends on body weight, caloric intake, and other factors.

As a result the Committee on Food and Nutrition of the National Research Council recommend the following daily "allowances" which allow a "margin of safety" for optimal nutrition.

MAN, 70 KG	CALORIES	THIAMINE, MG
Sedentary	2400	1.2
Moderately active	3000	1.5
Very active	4500	1.8
WOMAN, 50 KG		
Sedentary	2000	1.0
Moderately active	2400	1.2
Very active	3000	1.5

The recommended allowances for infants under 1 year of age is 0.4 milligram of thiamine per kilogram of body weight. Allowances for children range from 0.6 milligram of thiamine for children 1 to 3 years of age, to 1.2 milligrams for children at the age of 12 years. Active boys of 13 to 15 years of age should have about 1.3 milligrams of thiamine per day.

Although birds are very susceptible to a lack of thiamine in the diet, the poultry farmer is not required to give much thought to the thiamine content of his poultry rations. Good poultry rations usually contain more thiamine than the birds require. Starting chicks, up to 8 weeks of age, should receive about 0.9 milligram of thiamine per pound of feed. No requirements or allowances have been established for older birds.

National Research Council daily allowances for swine range from 1.4 milligrams of thiamine for pigs weighing 50 pounds,

to 4.2 milligrams for hogs weighing 250 pounds. Lactating sows and breeding boars should receive about 6.3 milligrams of thiamine per day. No allowances or requirements for thiamine have been established for ruminating animals (cattle and sheep).

Distribution of thiamine. Thiamine is quite widely distributed in foods and feed stuffs, but very few of our common foods can be considered extremely rich sources of this vitamin. The most potent natural sources of thiamine are yeast, cereal grains, and pork. Potatoes and other vegetables play an important role as a source of thiamine in spite of the fact that these foods are not rich in this factor. White flour, like polished rice, is a poor source of thiamine because most of the vitamin is lost in the refining process. As a result wheat bran is a fairly rich source of vitamin B₁. Raw milk is not a good source of thiamine, and the normal content may be reduced appreciably by pasteurization.

Thiamine is lost in varying amounts when vegetables are cooked. These losses vary according to cooking temperatures, type of food, and the amount of cooking water thrown away before the food is served. Ordinarily the loss of thiamine during cooking should not exceed 25 per cent, if foods are cooked properly in a minimum amount of water. Canning processes are not particularly destructive.

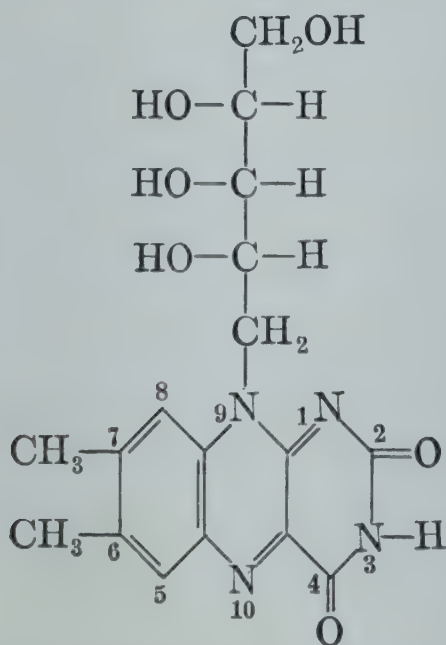
In many cases thiamine losses in vegetables may be greater during harvesting, handling, and storage than during actual processing in the cannery. Under normal conditions, however, blanching seems to be the most important single factor affecting vitamin losses in canned foods. Even canned goods must be stored at relatively cool temperatures (80° F or less) to prevent thiamine losses during storage. Freezing, per se, has little or no effect on thiamine. The greatest losses of thiamine in frozen foods occur prior to the freezing process or during the subsequent melting and cooking procedures. Good methods of dehydration may preserve as much as 75 to 85 per cent, whereas poor methods of dehydration may cause total destruction of the thiamine content of foods.

Riboflavin (B₂)

Riboflavin, the second member of the vitamin B complex, is sparingly soluble in water but differs from thiamine (B₁) by

being relatively stable to heat. It was first isolated from milk in 1879 as "lactochrome," a natural yellow fluorescent pigment. It was not until 1933 that Kuhn of Germany showed that the fluorescent pigment in milk was similar, chemically, to fluorescent pigments isolated from eggs and liver. All these pigments contained a flavinlike substance which accounted for the fluorescent properties. Consequently, these pigments were named lactoflavin (from milk), ovoflavin (from eggs), and hepatoflavin (from liver). Subsequently Kuhn and co-workers proved that the flavins from milk, eggs, and liver were identical compounds. In the meantime, Warburg of Germany had isolated a flavin-protein complex from yeast which he called "yellow enzyme" because it functioned as an oxidation catalyst.

Chemical studies in Holland, Germany, and Switzerland soon established the fact that the fluorescent pigment associated with Warburg's yellow enzyme was identical with the fluorescent pigment isolated from eggs, milk, and liver. This pigment was heat-stable, but it was readily destroyed by exposure to light. In the presence of alkali the rate of destruction by light was increased. Degradation studies led to the discovery that the pigment molecule consists essentially of a cyclic structure called isoalloxazine and a side chain which hydrolyzes to form a pentose sugar called ribose. The molecular configuration was confirmed by synthesizing the compound, which was found to have the following structure:



6,7-Dimethyl-9-D-ribylisoalloxazine

Riboflavin deficiency (aribo flavinosis). It is interesting to note that very little information was available regarding the physiological functions of riboflavin or the effects of riboflavin deficiency until the vitamin was isolated in pure crystalline form. It was then possible to initiate feeding tests and biological

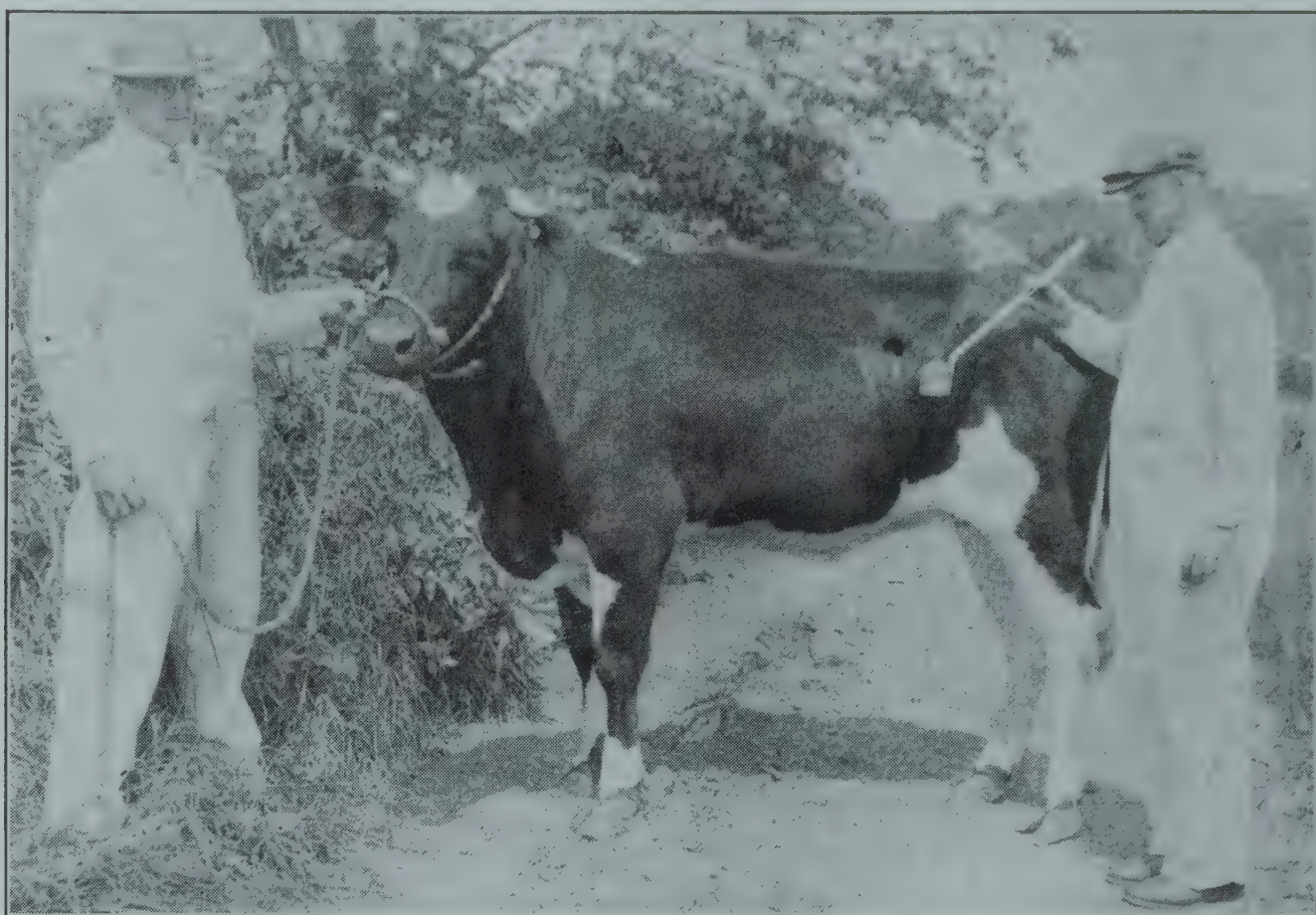


FIG. 16. Pennstate Homestead Jessie, showing permanent fistula in the rumen from which samples of the rumen contents were removed and studied. It was found that the rumen is capable of harboring microorganisms which possess the power to synthesize members of the vitamin B complex. (Data of Bechdel, Dutcher, Honeywell, and Knutsen.)

experiments in which riboflavin could be removed and added at will. These studies showed that riboflavin is an essential dietary factor for man and for most other animal species, such as rats, chicks, turkeys, dogs, and pigs. Workers at the Pennsylvania State College were the first to show that ruminating animals are able to obtain their requirements of riboflavin by bacterial synthesis in the rumen.

Riboflavin is essential for normal growth and good health. When riboflavin is omitted from the diet, certain characteristic pathological symptoms appear. Corneal vascularization (blood-

shot eyes) is one of the early symptoms. Monkeys develop a characteristic dermatitis (inflamed skin), and rats lose their fur as the result of a moist type of dermatitis.

Human beings develop characteristic lesions in riboflavin deficiency. This deficiency disease is known as ariboflavinosis. Cracks or fissures occur in the angles of the mouth. The lips show characteristic lesions (cheilosis). Occasionally lesions occur about the nose, ears, and eyes. Since pellagra is a multiple deficiency disease, most pellagrins are afflicted with the characteristic symptoms of riboflavin deficiency. The lesions of the mouth, lips, and eyes usually respond to riboflavin therapy, but the other symptoms of pellagra persist unless other dietary essentials are administered. People afflicted with riboflavin deficiency are sensitive to light (photophobia) and complain of blurred vision. These symptoms usually disappear when the patients are fed well-balanced diets containing adequate amounts of riboflavin.

Function of riboflavin. All existing evidence points to the fact that the pathological effects of riboflavin deficiency in animals occur as a result of disturbances in normal tissue respiration. We have learned in Chapter 8 that the normal processes of tissue metabolism, involving oxidative changes, depend on a complex chain of chemical reactions brought about by a similarly complex combination of interdependent enzyme systems. If any part of these interdependent enzyme systems fails to function properly, the efficiency of the whole system is affected adversely.

Consequently the characteristic lesions of riboflavin deficiency may be attributed directly or indirectly to a disturbance of normal oxidative changes in tissues as a result of the hampering of enzyme processes. If sufficient riboflavin is not available for the formation of the flavoproteins, the whole chain of oxidative reactions is affected.

Requirements. The daily requirement for riboflavin is related to body weight, to the amount of food ingested, and to metabolic activity. The daily allowance for adolescent and adult human beings is about 1.8 milligrams of riboflavin. Recommended daily allowances for optimal nutrition range from 1.5 to 3.0 milligrams, depending on age, sex, and physical activity. The

highest allowance (3.0 milligrams) is recommended for pregnant and lactating women.

Recommended allowances for domestic poultry are expressed in terms of milligrams of riboflavin per pound of feed rather than on the basis of daily intake. Starting chicks should receive

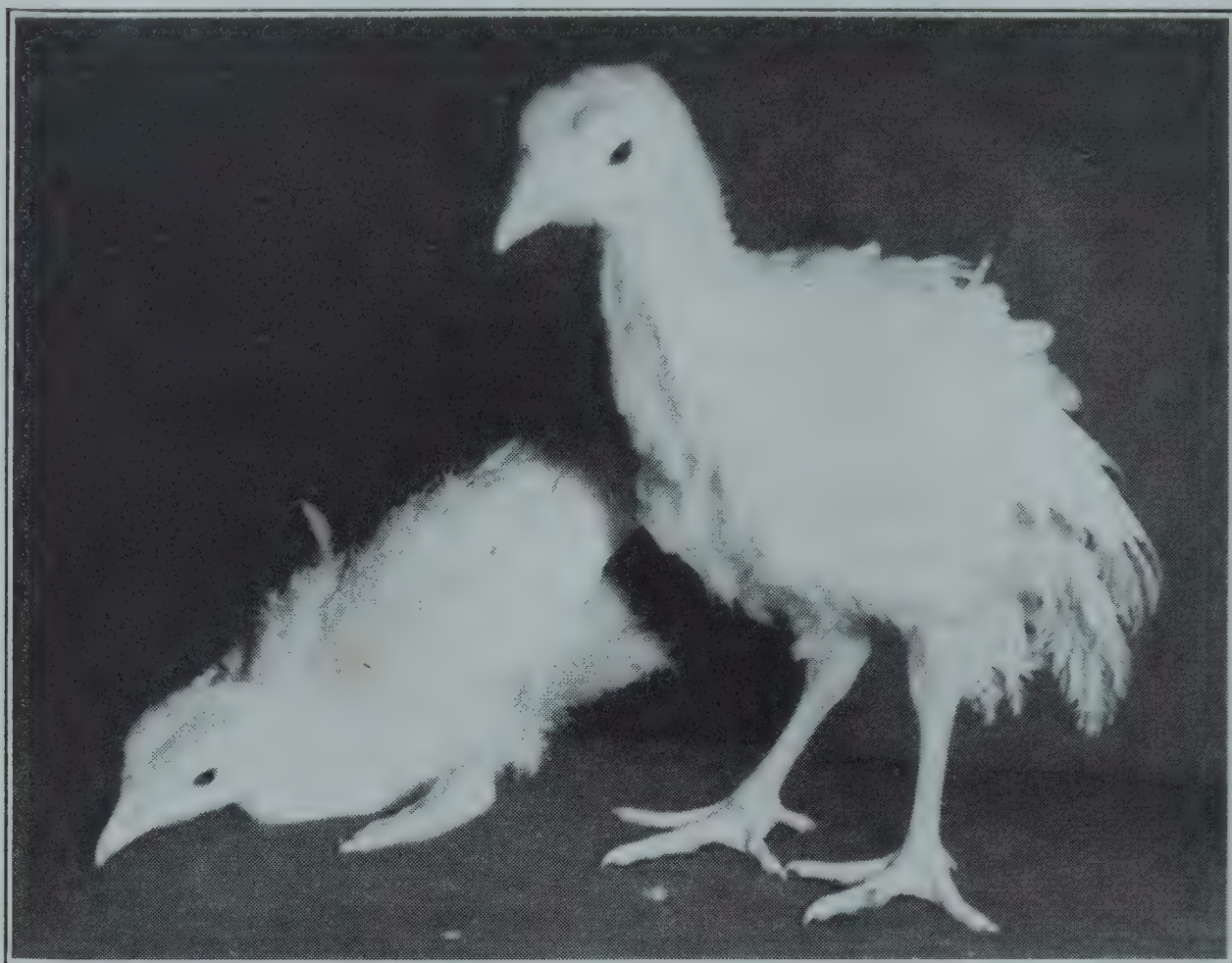


FIG. 17 Effect of the absence and presence of riboflavin in the ration of turkey poults. Note retarded growth and prostrated condition (leg paralysis and curled toes) of the poult which received the riboflavin-deficient ration in contrast to the poult which received the same ration supplemented with crystalline riboflavin. (Courtesy of Dr. R. V. Boucher.)

feed containing about 1.6 milligrams of riboflavin per pound of feed, and the allowance for laying and breeding hens is 0.9 and 1.3 milligrams, respectively. Most authorities agree that riboflavin plays a very important role in hatchability. A ration containing sufficient riboflavin for egg production may not be sufficiently rich in this vitamin to ensure maximal hatchability. Turkeys appear to have a higher requirement than chickens; poults and breeders requiring 2 and 1.6 milligrams, respectively, per pound of feed.

Daily riboflavin allowances for growing swine range from 2.1 milligrams (50 pounds live weight) to 6.3 milligrams (250 pounds live weight). No allowances have been recommended for breeding, pregnant, and lactating animals. Owing to the fact that ruminating animals are capable of synthesizing riboflavin by bacterial action in the rumen, the riboflavin content of the feed is not a matter of practical importance. The tentative allowance of riboflavin for horses is 2.0 milligrams per 100 pounds of body weight.

Distribution. Since riboflavin is necessary for carbohydrate metabolism, it is to be expected that riboflavin will be found in plant and animal tissues. The best sources of dietary riboflavin are yeast, liver, kidney, milk, powdered milk, whey, egg white, fish, oysters, and muscle tissues of beef, pork, and poultry. Cereal grains are fair but not rich sources. Probably the richest natural sources of riboflavin are fermentation residues from the manufacture of butyl and ethyl alcohols. Alfalfa meals are also relatively good sources of this vitamin.

Nicotinic acid

Nicotinic acid deficiency. Nicotinic acid and its amide, nicotinamide, are also known as *niacin*. This vitamin has been called the "antipellagra factor" because it is the key vitamin in preventing and curing characteristic skin and tissue lesions of people afflicted with pellagra.

Pellagra in humans is now considered to be a nicotinic acid deficiency disease, although other deficiencies are also likely to complicate the clinical picture. Pellagra is not uncommon in the southern states of the United States, and it is quite common in southern Italy. The low-income groups in both countries subsist on diets consisting largely of corn and corn products.

People afflicted with pellagra develop digestive disturbances characterized by gastroenteritis and diarrhea. In addition, the skin becomes red and inflamed, followed by a scaly condition and, finally, open sores. Usually this dermatitis is accompanied by a brown pigmentation. In the latter stages of the disease patients often become demented. Physicians who treat pellagrins

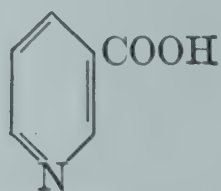
often refer to the clinical symptoms by using the expression "3-D's," which connotes diarrhea, dermatitis, and dementia.

When dogs are fed diets that produce pellagra in humans, the dogs develop a pathological condition known as *blacktongue*. Wisconsin workers discovered that nicotinic acid or nicotinamide will prevent and cure blacktongue in dogs. This work led to similar experiments with human pellagrins, with the result that nicotinic acid was found to play a specific role in the prevention of the characteristic skin lesions in pellagra.

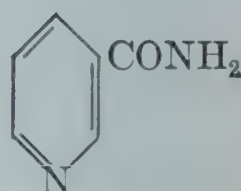
As a result of these experiments the antipellagric properties of nicotinic acid became quite firmly established. People afflicted with pellagra develop glossitis, a condition in which the tongue becomes swollen and fiery red in color, and ulcers often form beneath the tongue. The inflammation of the oral mucous membranes usually extends throughout the digestive tract. Relief of these symptoms is noticeable almost immediately when nicotinic acid is fed. Skin lesions, which are aggravated by exposure to sunlight, slowly disappear following nicotinic acid administration. Most clinicians describe dramatic recoveries when vitamin therapy is applied. Intestinal inflammation (enteritis) of swine is often a result of nicotinic acid deficiency.

Chemistry. The chemistry of nicotinic acid has been known since 1867 when Huber of Germany prepared it by the oxidation of nicotine. It was isolated by Funk in 1911 from rice polishings, but it was not recognized as an accessory food factor, since it failed to cure or prevent experimental beriberi. It was not until 1937 that nicotinic acid was recognized as a vitamin, as the result of the work on blacktongue in dogs and pellagra in man.

Nicotinic acid is pyridine-3-carboxylic acid or β -carboxypyridine. In medical practice it is usually administered as nicotinamide.



Nicotinic acid



Nicotinamide

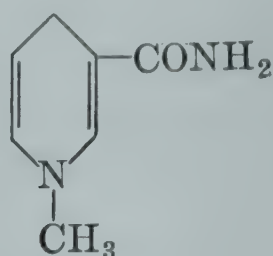
When it was discovered that nicotinic acid was destined to become an essential constituent of commercial vitamin preparations as well as of enriched foods, manufacturers suggested that a new term, *niacin*, be coined in order that this essential food factor might not be associated in the public mind with its less respectable parent, nicotine. Consequently the term niacin is often used to refer to nicotinic acid or to nicotinamide.

Nicotinic acid is dispensed as a white powder which is not hygroscopic and which is stable in air. It is soluble in alcohol-water mixtures but is not soluble in fat solvents. The amide has similar properties, but it is better tolerated in large doses than the free acid. The latter causes "hot flashes" and nausea when taken in large doses.

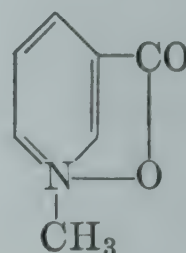
Physiology. Nicotinic acid plays its important physiological role in tissue metabolism by forming a necessary part of oxidative enzyme systems. Specifically nicotinic acid functions as a constituent of two coenzyme systems. These are known as coenzyme I and coenzyme II. Coenzyme I is known, chemically, as a diphosphopyridine nucleotide, which consists of a purine (adenine), nicotinamide, and two molecules of D-ribose phosphoric acid. Coenzyme II differs from coenzyme I in having three instead of two molecules of phosphoric acid. Details of the chemical properties of coenzymes I and II have been discussed in Chapter 8.

When nicotinamide is ingested in food or in proprietary preparations by humans, it is found that a portion is excreted in the urine as a fluorescent product, called N¹-methyl nicotinamide. It would appear that horses and guinea pigs do not methylate nicotinamide, since these species do not excrete appreciable amounts of N¹-methyl nicotinamide in the urine. Rats, on the other hand, do excrete this urinary metabolite. It is interesting to note that species that do excrete the methylated product are susceptible to large doses of nicotinamide. Species that do not excrete the methylated compound appear to be less susceptible to large doses of nicotinamide. The susceptibility to high doses is thought to be caused by excessive demethylation of the tissues. This is supported by the fact that methionine, which contains labile methyl groups, counteracts the toxic effects of nicotinic

acid. In other words, it is possible that excessive doses of nicotinic acid tend to deplete human and rat tissues of methionine, which is one of the essential amino acids. *Trigonelline*, another methylated product, is excreted in the urine. Recent researches have shown, quite conclusively, that L-tryptophan is a precursor of nicotinic acid. It is probable that intestinal microorganisms are responsible for the conversion of L-tryptophan to nicotinic acid.



N-methyl nicotinamide



Trigonelline

Requirements. Although all living organisms require nicotinic acid, minimum requirements for the various species have not been established. The recommended daily allowances for adult human beings range from 10 to 18 milligrams, depending on age, sex, and activity. Starting chicks should receive about 8 milligrams of nicotinic acid per pound of feed, but no recommendations have been made for laying and breeding hens. Daily nicotinic acid allowances for growing pigs range from 7 to 21 milligrams, depending on age and weight. Ruminating animals do not require dietary niacin.

Sources of nicotinic acid. Good food sources of nicotinic acid include such foods as beef and hog liver, yeast, kidney, lean meats, peanuts, salmon, eggs, milk, coffee and green vegetables.

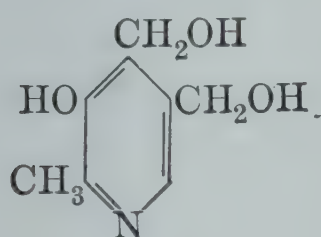
Pyridoxine (B₆)

This vitamin is one of the newer members of the water-soluble B complex. Its existence was first established about 1934, but it was not isolated in crystalline form until 1938. Proof for its chemical structure was first published in 1939.

Pyridoxine is known as vitamin B₆, *adermin*, *antidermatitis factor*, and *antiacrodynia factor*. Physicians have described a disease of infants in which a wet dermatitis covers the body, especially the legs and buttocks. This has been known as "pink disease" or "acrodynia." A similar disease can be produced in

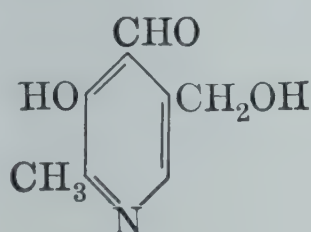
rats which is prevented and cured with pyridoxine. Not much is known regarding its role in human nutrition. Rats, dogs, and pigs develop epileptiform fits when subjected to pyridoxine-deficient diets. Since pellagra, ariboflavinosis, and beriberi are invariably multiple-deficiency diseases, humans afflicted with these diseases usually respond to pyridoxine therapy.

Chemistry. Two other physiologically related compounds are associated with pyridoxine, pyridoxal and pyridoxamine. It will be noted that the respective compounds are identical in structure with the exception of the chemical groups attached in the number 4 position of the pyridine ring.

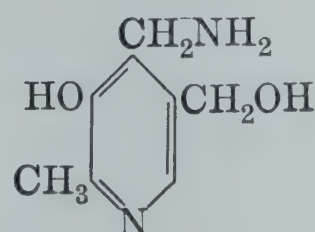


Pyridoxine

(2-methyl-3-hydroxy-
4,5-di(hydroxymethyl)-pyridine)



Pyridoxal



Pyridoxamine

Physiology. Not much is known regarding the functions of pyridoxine in metabolism. It is quite evident that pyridoxal phosphate serves as a coenzyme for at least five amino acid decarboxylases. Pyridoxal and pyridoxamine seem to be involved in tissue transamination. Pyridoxine, per se, does not seem to be involved in these reactions.

Requirements. No requirements have been established for man. There is evidence that the rat requires about 10 micrograms of pyridoxine per day, whereas the requirement of the chick is about 1.6 milligrams per pound of feed.

Distribution. Pyridoxine and its derivatives, pyridoxal and pyridoxamine, are found in egg yolk, wheat germ, yeast, liver, kidney, meat, milk, legumes, and whole grains.

Pantothenic acid

This vitamin, like other members of the vitamin B complex, owes its discovery to the important role that yeast has played in the development of vitamin research. In the very early days of vitamin investigation, Belgian workers had postulated the

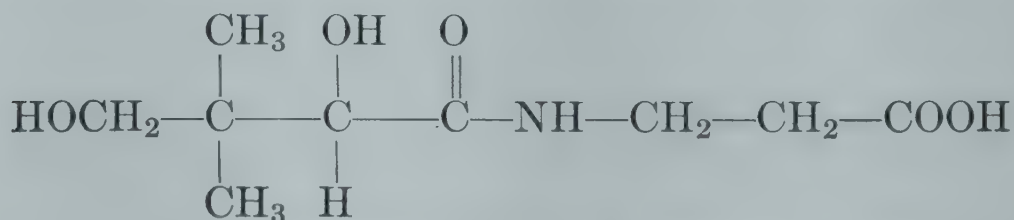
existence of a factor essential for the growth of yeast. They called this factor "bios."

R. J. Williams and associates finally isolated and identified the factor essential for normal multiplication of yeast cells during growth. During the progress of his investigations Williams found that this factor is present in practically all plant and animal tissues. Since it was necessary to isolate and identify the vitamin before its functions could be elucidated, we will discuss the chemical phases first.

Chemistry. By employing fractionation methods Williams was able to show that the yeast growth factor, bios, consists of two or more chemical substances. By means of adsorption techniques he was able to show that a soluble substance essential for yeast growth is not adsorbed on adsorbing agents but passes through into the filtrate. The substance became known as the *filtrate factor*. During the early work concentrates of the filtrate factor were shown to prevent and cure dermatitis in chicks. Consequently the substance was often called the *chick antidermatitis factor*. It was found that black rats developed gray hair on diets deficient in the filtrate factor. Since the graying of the hair is called "nutritional achromotrichia," some writers referred to the substance as the *antiachromotrichia factor*.

Williams and co-workers finally used electrolytic methods to separate the crude filtrate product into two fractions. One was acidic and the other was basic in nature. The acidic fraction was found in extracts of all plants and animal tissues studied. Consequently Williams postulated the existence of a definite chemical substance, which he named *pantothenic acid*, from the Greek word meaning "everywhere."

By 1939 Williams and co-workers were able to announce the synthesis of pantothenic acid, confirming the chemical structure postulated in the previous year. It is actually a dipeptide formed by the union of alanine with a derivative of butyric acid.



Pantothenic acid
(2,4-dihydroxy-3,3-dimethylbutyryl- β -alanine)

Pantothenic acid is soluble in water and unstable to heat. It is sold on the market in the form of calcium pantothenate. Although it is evident that pantothenic acid is essential for all living organisms, its physiological role is still in doubt. Chick dermatitis, affecting eyes, mouth, and toes, responds to pantothenic acid feeding. Fox breeders find that the feeding of this vitamin helps in preventing the graying of the fur, and recent reports from the University of Wisconsin indicate that riboflavin also plays an important role in maintaining normal color of fox fur. Pantothenic acid is thought to be necessary for the nutrition of the intestinal microorganisms which, in turn, have the power under proper conditions to synthesize certain of the other water-soluble vitamins.

Requirements. No requirements have been established for animal species other than poultry and rats. Poultry requirements range from 2.5 to 5.0 milligrams per pound of feed for growth and reproduction. Requirements for ducks and turkeys are somewhat higher than those for chickens.

Distribution. Pantothenic acid is quite well distributed in plant and animal tissues. Good food sources include egg yolk, liver, kidney, milk, milk whey, yeast, rice bran, and a number of cereals and vegetables.

Biotin

This vitamin was originally a part of the so-called "bios complex." It is also known as *vitamin H*, the *anti-egg white injury factor*, and *coenzyme R*. During the early work on bios, this factor was found in the fraction adsorbed on charcoal. Since diets deficient in this factor caused an eczematous dermatitis in rats, it was called vitamin H (from the German word *Haut*, meaning skin). In 1939 biotin was found to be identical with coenzyme R, which was isolated from a legume nodule organism.

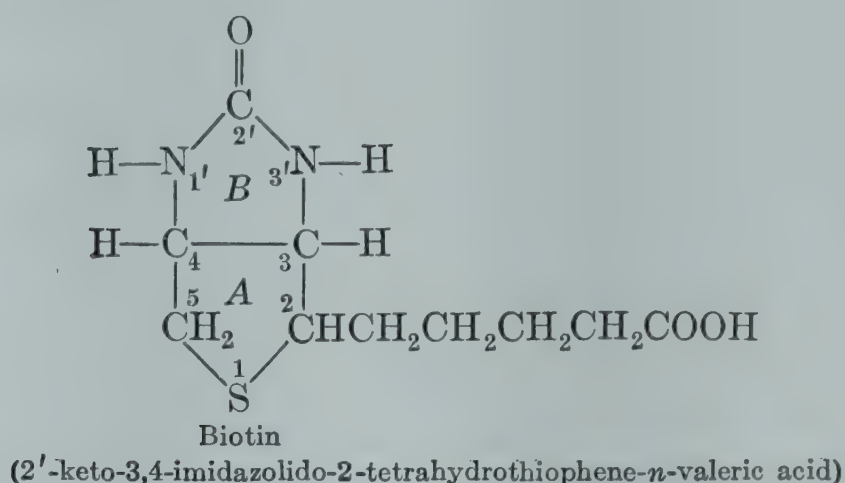
Deficiency symptoms. A deficiency of biotin produces eczematous dermatitis in rats, which is accompanied by alopecia (loss of hair) and edema of the tissues, particularly of the paws.

When rats are fed a diet in which egg white is the sole source of protein, biotin deficiency occurs. This egg white injury is caused by an *antivitamin* in egg white called *avidin*. Avidin

is a protein complex which unites with biotin in the diet, inactivating the biotin and rendering it unavailable.

Biotin deficiency in chicks and turkeys results in a characteristic dermatitis similar to that of the rat. Humans are susceptible to biotin deficiency and complain of stomach ache, lassitude, muscular pains, and loss of appetite. Skin changes are also observed.

Chemistry. The determination of the structure of biotin and its synthesis were largely the work of du Vigneaud and co-workers. Biotin consists of an eight-membered ring. A portion of the ring consists of a thiophene (*A*) nucleus, and the other portion consists of a urealike structure (*B*), called the imidazole nucleus. The latter contains nitrogen in the ring, and sulfur is a constituent of the former. A valeric acid side chain completes the structure.



Function. Not much is known regarding the physiological functions of biotin. A large number of microorganisms require it for growth. It is said that the potency of biotin is so great that 1 gram in 250 million gallons of water is sufficient to supply the requirements of yeast and certain bacteria. Some bacteria can synthesize biotin. Some evidence exists to indicate that biotin is essential for the larval development of insects.

Requirements. We have stated that biotin is required by some microorganisms and by birds, rats, and man. Ruminants are capable of synthesizing biotin in the rumen by means of rumen microflora. Man responds to doses of 50 to 300 micrograms of biotin per day. One pound of ration should contain

from 0.045 to 0.070 milligram of biotin when fed to chicks, and turkey-poult rations should contain somewhat smaller amounts.

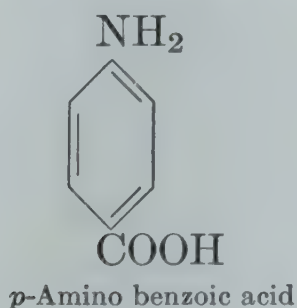
Distribution. Biotin occurs in many plant and animal tissues, among which the following may be considered rich sources: liver, kidney, eggs, yeast, milk, vegetables and nuts.

p-Amino benzoic acid

Although the organic chemist has been familiar with this relatively simple chemical compound since 1863, it was not until 1940 that it was recognized as an accessory food factor. In that year it was shown that *p*-amino benzoic acid (PABA) neutralizes the harmful effect of sulfa drugs. As a result it was postulated that PABA is essential for certain intestinal bacteria and that the bacteriostatic action of the sulfonamides is due to physiological competition between two structurally similar compounds (PABA and sulfonamide) for combination with an essential enzyme needed for normal metabolism.

Some evidence exists to indicate that PABA is essential for normal rat and chick nutrition. Apparently it is needed by some microorganisms but can be synthesized by others. Recent work shows that PABA is a constituent of the folic acid molecule. For a time PABA was called the "achromatrichia" or "antigray hair" factor, but most laboratories have been unable to show that this vitamin is very specific in affecting pigmentation of hair. Efforts to prevent graying of human hair by feeding anti-gray hair factors have been unsuccessful.

Chemistry. *p*-Amino benzoic acid is a white crystalline powder which is soluble in water. As the name indicates, the amino group is attached to the benzene ring in a position para to the carboxyl group. The competition of PABA and sulfanilamide for certain enzyme molecules can be explained by noting the similarity of chemical structure of these compounds.



Function. Like pantothenic acid PABA is probably essential for the growth of certain intestinal bacteria, which, in turn, are capable of synthesizing the other vitamins. In this sense PABA is necessary for intestinal synthesis of other vitamins. Recent studies on rickettsial infections, such as Rocky Mountain spotted fever, indicate that PABA is useful in the clinical treatment of these diseases.

Requirements. No official requirements have been recommended. Chicks are supposed to require 30 milligrams of PABA per 100 grams of ration, and some writers state that 0.75 milligram is required to prevent nutritional achromotrichia in rats.

Distribution. It is possible that any good source of the B complex vitamins will also be a good source of *p*-amino benzoic acid. Of these sources, yeast, rice polishings, wheat germ, and molasses are the best.

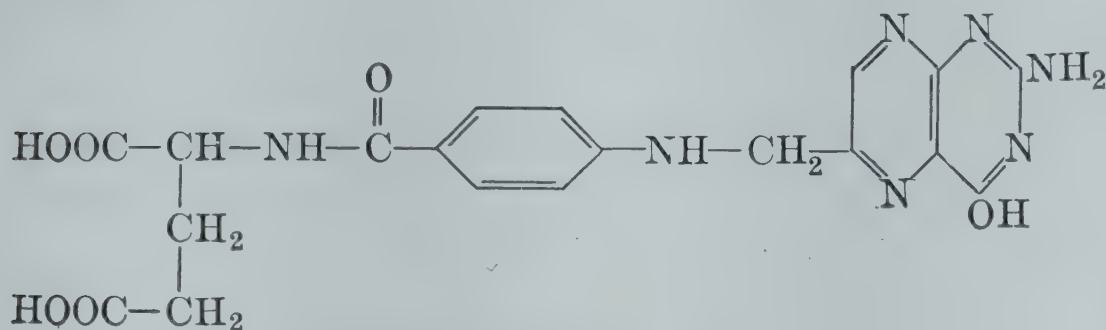
Folic acid

This vitamin has appeared in the literature under a variety of names. In 1938 Almquist postulated the existence of an unknown factor present in middlings and yeast which was essential for chicks. Hogan and Parrott (1939), working with chicks, found that they could prevent and cure macrocytic anemia in chicks with a liver extract. They postulated the existence of an unknown member of the vitamin B complex and suggested that it be called *vitamin B_c* to indicate that it belongs to the B complex and that it is specific for chicks.

Snell and Peterson (1940) used Norit to adsorb a material from yeast extracts. One of the Norit eluates contained a substance which stimulated the growth of *Lactobacillus casei*. This factor was called the *Norit eluate factor* and, later, the *L. casei factor*. Stokstad and Manning called it *factor U*. Wisconsin workers, using *Streptococcus faecalis* and *L. casei* organisms, found that extracts of many leafy plants contain a stimulating acid, for which they suggested the name *folic acid* (from leaves). Day had also used the term *vitamin M* to designate a fraction of liver extract which prevented and cured nutritional macrocytic anemia in monkeys. As work progressed, evidence accumulated to prove that all these factors are identical.

Chemistry. Workers from the Lederle Laboratories, Inc., and Calco Division of the American Cyanamide Company identified and synthesized the *liver L. casei factor* and the *fermentation L. casei factor* which, by common consent, are now known as folic acids. Chemically, folic acid has been identified as pteroylglutamic acid, a bright yellow substance which is unstable in acid media. Folic acid is also affected by light.

The pteroylglutamic acid molecule is composed of (1) a two-ringed nitrogen compound (pteridine), (2) *p*-aminobenzoic acid, and (3) glutamic acid. When combined they form folic acid, a dipeptide.



Folic acid (pteroylglutamic acid)

Actually there is evidence to show that there are a number of compounds in what might be termed the folic acid group. These may differ slightly in chemical structure. For example, the liver *L. casei* factor is pteroylglutamic acid. The fermentation *L. casei* factor (obtained from the anaerobic fermentation of a diphtheroid type of organism) is known to be pteroyltriglutamic acid or pteroyldiglutamylglutamic acid. Another member of the folic acid group contains seven glutamic acid residues and is known as pteroylhexaglutamylglutamic acid. This compound (which is obtained from yeast) is known in the literature as the *B_c conjugate*. Since the conjugate is a peptide, the enzyme which hydrolyzes the peptide is known as the *B_c conjugase*. The conjugate yields pteroylglutamic acid (folic acid) and free glutamic acid, upon hydrolysis.

Function. The simple compound, pteroylglutamic acid, is active for *L. casei*, *S. lactis* R., the chick, the rat, the monkey, the turkey, and the guinea pig. The triglutamic acid derivative seems to function as efficiently as the monoglutamic acid compound. The heptaglutamic acid compound, however, is relatively unavailable in the absence of the conjugase enzyme. Undoubt-

edly this enzyme is present in chick and rat tissues but absent from certain microorganisms, since the conjugate is utilized quite well by the former but quite inefficiently by the latter.

Folic acid prevents and cures a characteristic anemia in rats, chicks, monkeys, and humans. It is essential for lactation in rats and hatchability of eggs as well as for normal growth and health of turkeys, chicks, and guinea pigs. Clinical studies indicate that folic acid is effective in treating macrocytic anemias of pregnancy, sprue, pellagra, and certain types of pernicious anemias.

Requirements. No requirements have yet been established. Folic acid is toxic in large doses (500 to 5000 times the therapeutic dose).

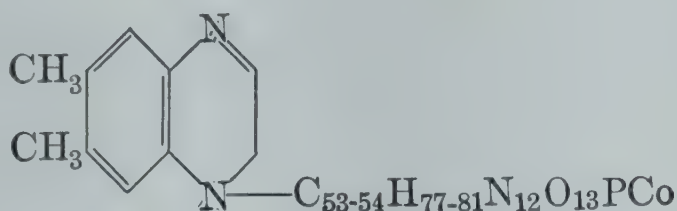
Distribution. Good sources of folic acid include liver, yeast, kidney, beef, veal, green leafy vegetables, and some cereal products, especially wheat.

Vitamin B₁₂

In 1948, workers at Merck and Company announced the isolation of a new antipernicious anemia factor from liver to which they have tentatively assigned the symbol B₁₂. This factor has been isolated as a red crystalline material which can be taken orally, thereby eliminating the annoyance and expense of taking frequent injections of liver preparations. It promises to be valuable in the treatment of pernicious anemia. Vitamin B₁₂ and folic acid appear to play closely interrelated roles. It appears that a compound called *thymidine* is needed for the manufacture of nucleic acids for the construction of new blood cells (reticulocytes). Folic acid makes possible the increased synthesis of *thymine*, which is necessary for the synthesis of thymidine. Vitamin B₁₂ possesses the property of catalyzing the production of new blood cells when adequate amounts of thymidine are available. Medical authorities feel that these antianemia factors may play an important role in the prevention and cure of pernicious anemia. Vitamin B₁₂ is thought to be a constituent of the *animal protein factor*.

Although the chemistry of vitamin B₁₂ has not yet been established with certainty, Folkers of Merck and Company has presented evidence indicating that the red crystalline vitamin

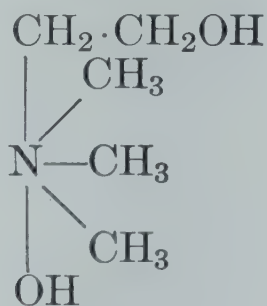
has a minimum molecular weight of 1300. Degradation studies indicate that the molecule contains 5,6-dimethylbenzimidazole. The B₁₂ molecule contains cobalt (4.5 per cent), phosphate, and a fraction which has not yet been identified. The following provisional structure has been assigned to vitamin B₁₂.

Vitamin B₁₂

Choline

Although choline is usually included in the list of water-soluble vitamins, some workers feel that it should not be classified as a vitamin because the amounts usually required are extremely large compared with requirements of the recognized accessory food factors.

Chemistry. Choline, a constituent of lecithin, has been known for many years. It is a derivative of ammonium hydroxide, as shown in the following formula:



Choline
(hydroxyethyltrimethyl ammonium hydroxide)

Function. The principal biochemical role of choline in tissue metabolism is to furnish methyl groups for transmethylation processes. If the essential amino acid, methionine, is not available in the diet, choline can donate methyl groups to the nonessential amino acid, homocystine, which in turn can be converted to methionine. Details regarding this reaction are discussed in Chapter 22.

Choline serves as a constituent of many phosphatides and phospholipids. Acetylcholine functions in the reduction of blood

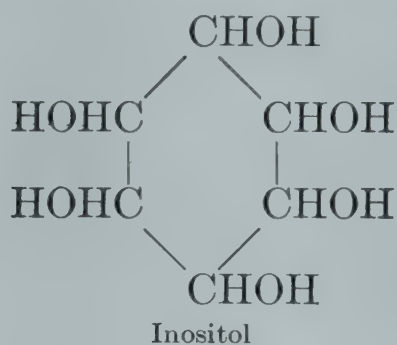
pressure and is involved in the transmission of nerve impulses. Choline is also involved in the regulation of fat metabolism. A disease of poultry, known as *perosis* or *slipped-tendon disease*, responds to a number of dietary factors, including choline.

Requirements. Young growing animals require more choline than mature animals. Dogs require about 35 milligrams of choline per kilogram of body weight, and the daily requirements of rats and chicks are about 20 milligrams and 75 milligrams, respectively. No requirements have been established for man.

Distribution. Natural sources of choline include liver, heart, kidney, sweetbreads, egg yolk, tongue, yeast, meats, cereals, and leafy vegetables.

Inositol

The chemistry of inositol has been known for many years. Chemically, it is a hexahydroxycyclohexane.



Inositol is a stable compound soluble in water. On account of its hydroxyl radicals, it readily forms esters with acids, particularly with phosphoric acid. To date there is little evidence regarding its biological functions, since the mouse is the only animal for which inositol has been found to be essential. Inositol-deficient mice develop alopecia (loss of hair). There is some evidence to show that this compound has a lipotropic function; i.e., it prevents the deposition of fat in liver and other body tissues. Good sources of inositol include whole grain cereals, wheat bran, and yeast.

Citrin (vitamin P)

In 1936, Szent-Györgyi postulated the existence of a substance in lemon peels and Hungarian peppers that tends to strengthen

blood capillary walls and prevent hemorrhages. Prior to this time, the effect of Hungarian peppers and citrus fruits on capillary permeability was attributed to vitamin C. This new substance was called *vitamin P* because it was supposed to affect vascular permeability.

Later work indicated that vitamin P consists of two flavone dyes, thought to be glucosides of hesperidin and eriodictyol. According to Szent-Györgyi the active substance is the hesperidin glucoside, which he named *citrin*.

Workers are not in complete agreement regarding the chemical identity of citrin. Evidence is accumulating to indicate that a group of flavone glucosides do possess the property of preventing capillary fragility. A new member of this flavone family is rutin, which has been isolated from the leaves of several plants, particularly buckwheat and tobacco. Hesperidin has been isolated from fruits (grapes, citrus fruits, and prunes).

In April 1950 a Joint Committee on Biochemical Nomenclature of the American Society of Biological Chemists and the American Institute of Nutrition recommended that the term “‘vitamin P’ should no longer be employed” since its identity as “a substance of a vitamin nature has not been established.” It is felt that the application of the term “vitamin P” to members of a group of polyphenolic substances will lead only to confusion.

Vitamin C (ascorbic acid)

Scurvy is a disease known and described since the twelfth or thirteenth century. The disease was most common among sea-faring men, soldiers in invading armies, crusaders, explorers, people in asylums, prisoners in penitentiaries, and populations living in famine-stricken areas. In all cases the development of the disease was associated with the lack of fresh foods, particularly fruits and vegetables.

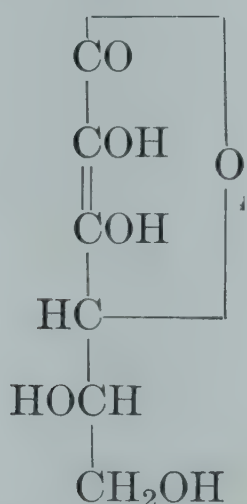
Although many theories have been advanced regarding the underlying causes of scurvy, it was not until 1895 that it became possible to produce the disease experimentally. In that year Theobald Smith described the production of scurvylike lesions in guinea pigs by feeding a diet of oats and hay. During the

years 1907 to 1912 Holst and Frolich used Smith's guinea pig method and discovered that the scurvy lesions could be prevented and cured by feeding fresh cabbage and fresh berries. They also proved that the curative effects of these fresh foods could be destroyed by heat and oxidation with air.

As a result of subsequent research in American laboratories the conviction grew that scurvy could be prevented and cured by a vitaminlike material contained in fresh fruits and vegetables. Citrus fruit juices were found to be most potent in this respect. Consequently the existence of vitamin C was postulated. This became known as the *antiscorbutic vitamin* (from the medical term "scorbutus," meaning scurvy). Although vitamin C is a water-soluble vitamin, it should be emphasized at this point that this vitamin is not a member of the water-soluble vitamin B complex.

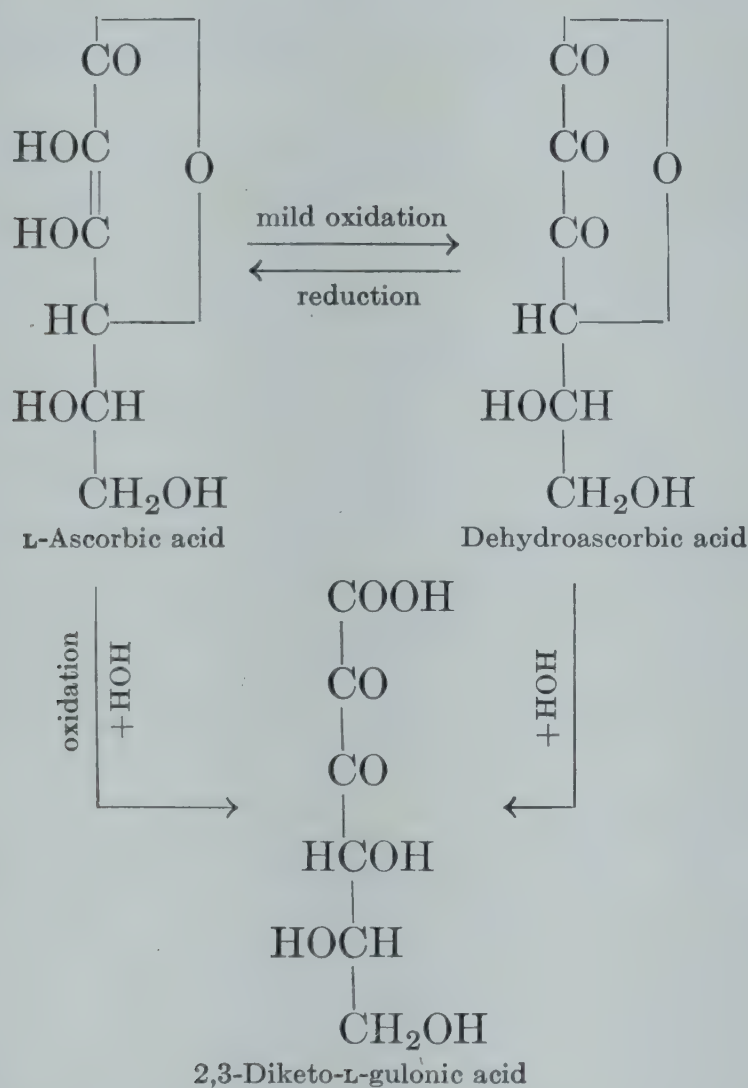
Pathology. Scurvy in humans or guinea pigs is characterized by fragility of capillary walls throughout the body, resulting in muscular and internal hemorrhages. Teeth become loose, bones become fragile and bacterial invasion of tissues, particularly of dental tissues, follows vitamin C deprivation. Pyorrhea of the gums and dental caries seem to be associated with a lack of the antiscorbutic factor.

Chemistry. Vitamin C is a white crystalline acid containing six carbon atoms. It is closely related, chemically, to the hexose sugars. In the early stages of the research work on this substance, writers referred to it as hexuronic and glucuronic acid. The acid exists as a lactone and has the following configuration:



Vitamin C (ascorbic acid)

Since the pure crystalline vitamin cures and prevents scurvy, Szent-Györgyi named it *ascorbic acid*. For a time this name was not accepted by the American Medical Association, and they pressed for the adoption of the term *cevitamic acid*. Ascorbic acid, however, became the official chemical designation for vitamin C. Ascorbic acid, which exists in the reduced form, is easily oxidized and reduced. When subjected to mild oxidation, ascorbic acid forms dehydroascorbic acid, which is as active, physiologically, as the parent acid. Under less mild oxidation, 2,3-diketo-L-gulonic acid is formed. This compound will not cure or prevent scurvy. These oxidation steps are as follows:



Ascorbic acid is synthesized commercially and sold in enormous volume at a price that competes favorably with natural products containing vitamin C. Because of its ease of oxidation ascorbic acid has found use in the food industry as an antioxidant.

Function. Vitamin C is synthesized by the higher plants and by many of the simpler forms such as molds and bacteria. It is

also synthesized by seeds during germination. Men, monkeys, and guinea pigs must receive this vitamin in the diet; other species, such as rats, horses, and ruminants, are able to synthesize ascorbic acid. Apparently this synthesis is not microbial in origin but is a step in the complex processes of tissue metabolism.

Ascorbic acid has a definite role in the formation of intercellular substance (collagen), which is essential for maintenance of normal tissue structure. In the absence of ascorbic acid, teeth soften, dentine formation is hampered, and normal tooth structure disappears. Bone formation and repair depend on vitamin C, and the healing of tissue wounds improves with vitamin C therapy. Some evidence points to the fact that ascorbic acid tends to increase resistance to certain infectious diseases of bacterial origin.

No one has been able to show that ascorbic acid forms a part of tissue respiration systems. Whatever its action, it is quite evident that this vitamin plays an important role in tissue physiology. Evidence is accumulating which indicates that ascorbic acid may occur in some type of coenzyme system.

Requirements. Although 10 to 20 milligrams per day will usually prevent the development of scurvy symptoms in humans, the recommended daily allowances for optimal nutrition are as follows: adults, 70 to 75 milligrams; pregnant and lactating women, 100 to 150 milligrams; children to 12 years of age, 30 to 75 milligrams. Species other than men, monkeys, and guinea pigs do not require dietary ascorbic acid but seem to be able to synthesize it during metabolism.

Distribution. Natural foods that are relatively rich in ascorbic acid include the following: acid fruits (citrus fruits, rhubarb, tomatoes), peppers, cabbage, and leafy vegetables. Rose hips and many berries are also excellent sources.

Stability. Owing to its chemical nature, ascorbic acid is very susceptible to oxidation. It is preserved quite readily in acid media, but it disappears rapidly when heated in neutral or alkaline media. Certain respiratory enzymes destroy vitamin C. Consequently losses of ascorbic acid during fresh storage are appreciable. Blanching destroys oxidizing enzymes and tends to preserve the vitamin C content of processed foods. Dehydrated foods are characterized by low retentions of vitamin C.

FAT-SOLUBLE VITAMINS

Members of this group of accessory food factors are characterized by being soluble in fat solvents, such as diethyl ether, petroleum ether, and chloroform. Consequently they are found in nature closely associated with those tissues that tend to store fats and oils.

Vitamin A

The existence of this vitamin was first suspected in 1912–1913 when Osborne and Mendel of Yale University observed an eye disease (ophthalmia) in rats which could be prevented and cured by feeding small amounts of cod-liver oil. Concurrently McCollum of the University of Wisconsin found that butter fat stimulated growth in rats. When olive oil replaced butter fat in the purified experimental diet, rats failed to grow and developed ophthalmia, as described by the Yale investigators. McCollum saponified butter fat with alkali, thereby changing the butter fat to water-soluble glycerol and soaps. When olive oil was mixed with the saponification mixture and removed with ether and fed to experimental rats, McCollum found that the olive oil stimulated growth. He reasoned that a fat-soluble growth promoting factor had been transferred from the saponified butter fat to the non-growth-promoting olive oil. Consequently he postulated the existence of a new factor and suggested that it be called *fat-soluble A*.

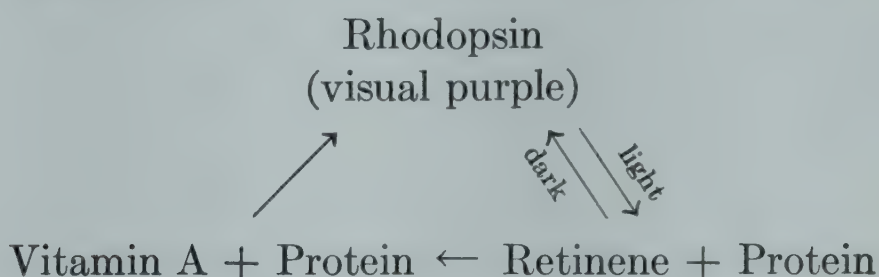
Vitamin A deficiency. When diets are deficient in vitamin A, human beings become afflicted with night blindness (nyctalopia), harsh voices, rough scaly skin, and dry eyelids, owing to lack of normal secretion of the tear glands. In later stages the eyelids (conjunctivae) become inflamed, and secondary infection usually follows. Occasionally, ulcers form on the eyeball (cornea), and blindness may result.

Respiratory infections are common in vitamin A deficiency, owing to changes in the epithelial linings of the respiratory tract, which permit bacterial invasion of tissues. Intestinal inflammation, sterility, and formation of kidney stones (renal calculi) have also been observed in vitamin A deficiency.

When vitamin A-deficient rats receive butter fat or cod-liver oil before pathological changes have become irreparable, symptoms disappear, growth is resumed, reproduction occurs, and the animals appear to be normal. Post-mortem examination reveals the presence of scar tissue, showing that the vitamin deficiency lesions have been repaired.

Functions of vitamin A. Histological studies on tissues of vitamin A-deficient animals show a series of abnormal changes (keratinization) in epithelial tissues. Bottle-shaped mucous-secreting cells become flat and degenerate. The protective action of normal secretions is lost, and secondary infections obtain a foothold.

Night blindness is a direct result of an insufficient vitamin A supply. The retina of the eye contains a pigment known as *rhodopsin* (or *visual purple*), which functions to change the energy of light into nerve impulses. Rhodopsin, which is bleached by light, is regenerated in the absence of light. Chemically rhodopsin is a protein-vitamin A complex which bleaches to a light yellow compound called *retinene* or *vitamin A aldehyde*. Retinene, in turn, breaks down to protein and vitamin A. The protein then unites with vitamin A reserves to resynthesize rhodopsin. The operation of this system may be shown schematically as follows:



Relation of carotenoid pigments to vitamin A. Since our knowledge of the chemistry of vitamin A was a direct result of researches on the carotenoid pigments, it is necessary to review some of the early work on these important compounds. By 1919 very little was known regarding the chemical properties of vitamin A, other than the fact that it seemed to be associated with the unsaponifiable fraction of certain animal fats and oils.

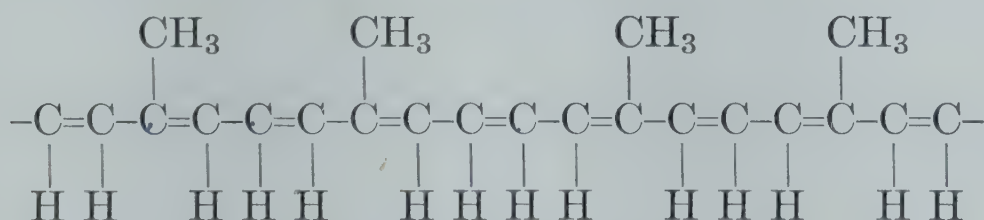
During that year (1919) Steenbock, of the University of Wisconsin, called attention to the fact that pigment-rich foods seem to possess growth-stimulating properties similar to those

obtained when butter fat and cod-liver oil are fed. Solvent extraction studies on these pigmented foods (carrots and leafy plants) led to the conclusion that the fat-soluble carotenoid pigments are responsible for the growth-stimulating properties.

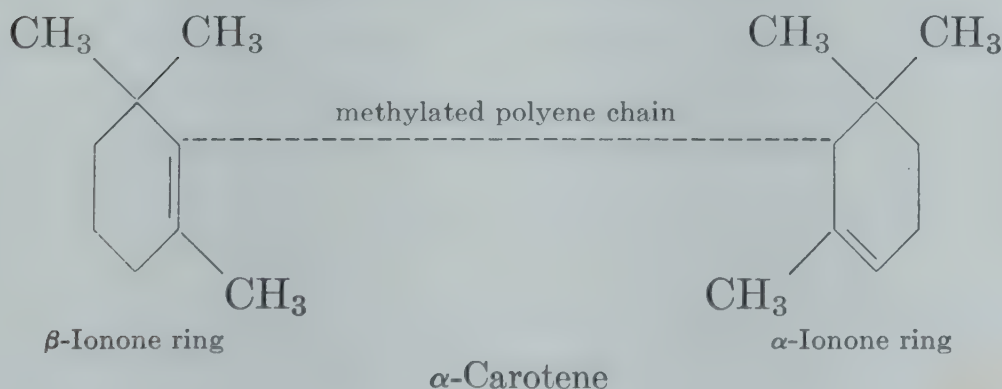
In 1927 Von Euler of Sweden was able to show that crystalline carotene possesses vitamin A-like properties when fed to vitamin A-deficient rats. Moore of England (1929) was able to prove that purified crystalline carotene is a provitamin or precursor of vitamin A and that the body tissues have the ability to change carotene into vitamin A for physiological purposes.

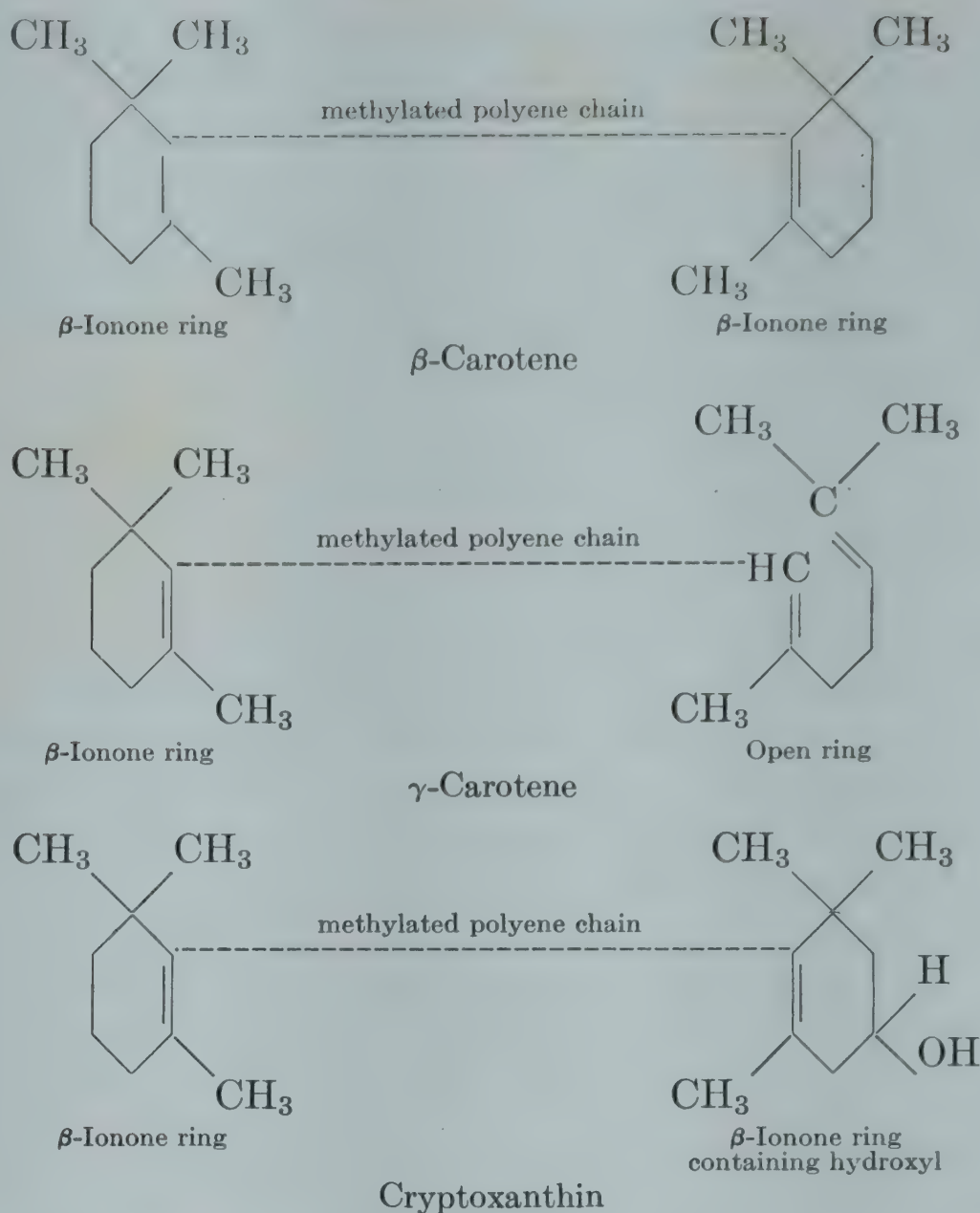
Chemistry. Carotene is an orange-colored compound that forms orange or yellow solutions in petroleum ether, depending on the concentration. Karrer and Kuhn showed that the hydrocarbon, carotene ($C_{40}H_{56}$), consists of a polyene chain containing eighteen carbon atoms and nine double bonds. Subsequent research showed that there are a number of carotenoid pigments and that these pigments differ in the type of chemical groups that are attached to the ends of the polyene chain. These terminal structures consist of ionone rings or closely related open rings. When the carotene molecule contains two β -ionone rings, the substance is called β -carotene. If one ring is β -ionone and the other is α -ionone, the compound is called α -carotene.

The following formulas are self-explanatory. The methylated polyene chain, which is common to the carotenoids, is shown in the following structure:



Methylated polyene chain
(common to the following carotenoids)



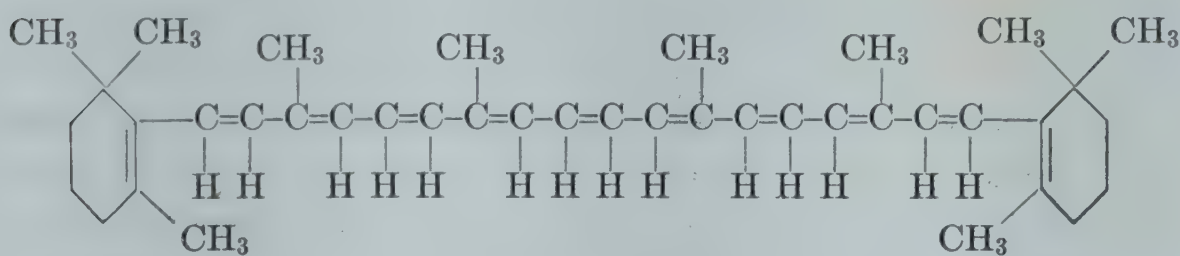
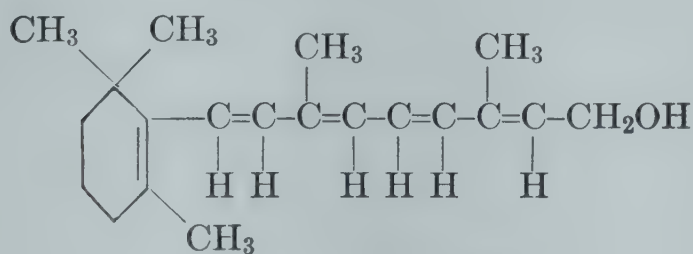


Another carotenoid pigment, lycopene, consists of the methylated polyene chain to which are attached two open structures. Lycopene is the characteristic red pigment of the tomato.

From the standpoint of nutrition, β -carotene is the most important of the carotenoid pigments. It is roughly twice as potent as the α -, γ -, or cryptoxanthin pigments. Lycopene possesses no vitamin action. When β -carotene is ingested, the body tissues (in all probability the intestinal mucosa) transform the pigment into vitamin A. It is for this reason that the carotenes are called *provitamins A* or *precursors of vitamin A*. The efficiency of this conversion varies with different animal species.

As soon as the chemical structure of β -carotene was established, Karrer postulated the chemical structure of vitamin A. Later work on vitamin A concentrates prepared from fish-liver

oils established the correctness of Karrer's hypothesis. Authorities are now agreed that vitamin A is semi- β -carotenol. When β -carotene is converted to vitamin A in the body, the polyene chain is broken at the central double bond followed by the formation of primary alcohol groups on the terminal carbons of each portion of the broken polyene chain. As a result two molecules of vitamin A should be formed from one molecule of β -carotene, as shown in the following formulas:


$$\beta\text{-Carotene (C}_{40}\text{H}_{56})$$


Vitamin A ($C_{20}H_{29}OH$)
(semi- β -carotenol)

Although theory calls for the formation of two molecules of vitamin A from one molecule of β -carotene, physiological evidence indicates that, weight for weight, the latter is about one-half the biological value of the former. Recent studies by Glover, Goodwin, and Morton of England indicate that β -carotene is not split by simple hydrolytic fission to form two molecules of vitamin A. These workers believe that random oxidation of β -carotene yields but one molecule of vitamin A aldehyde (retinene) and that this aldehyde is rapidly reduced by tissue enzyme systems to vitamin A alcohol. This theory seems to agree with the biological values mentioned above.

Vitamin A is found in the tissues and blood stream as an ester of fatty acids. Some vitamin A may be present as the free alcohol. The vitamin is now obtainable on the market as crystalline vitamin A alcohol and in the form of crystalline esters, of which vitamin A acetate is probably the most important.

Evidence has accumulated to indicate that vitamin A may exist in two forms, i.e., vitamin A₁ and vitamin A₂. Vitamin A₁ refers to the factor found in marine fish-liver oils, whereas vitamin A₂ is found in fresh-water fish. It is evident that vitamins A₁ and A₂ differ slightly in chemical constitution, but the chemistry of vitamin A₂ is still in doubt. The term, vitamin A, as used in this chapter refers to the usual form of the vitamin as found in marine fish-liver oils, namely, vitamin A₁.

Vitamin A is relatively unstable in the presence of air or oxidizing agents. Stabilizing agents, known as antioxidants, have been used to preserve vitamin A in certain types of foodstuffs and in many pharmaceutical preparations. The most common commercial sources of vitamin A are the fish-liver oils. Preparations of high potency can be prepared by separating the vitamin from unsaponifiable residues of fish-liver oils. The most potent preparations, however, are made from fish-liver oils by molecular distillation. There is evidence that the carotenes and vitamin A undergo molecular rearrangements resulting in the formation of isomers which may not possess the physiological properties of the parent substance.

Requirements. Recommended daily allowances for human beings range from 1500 International Units for infants to 5000

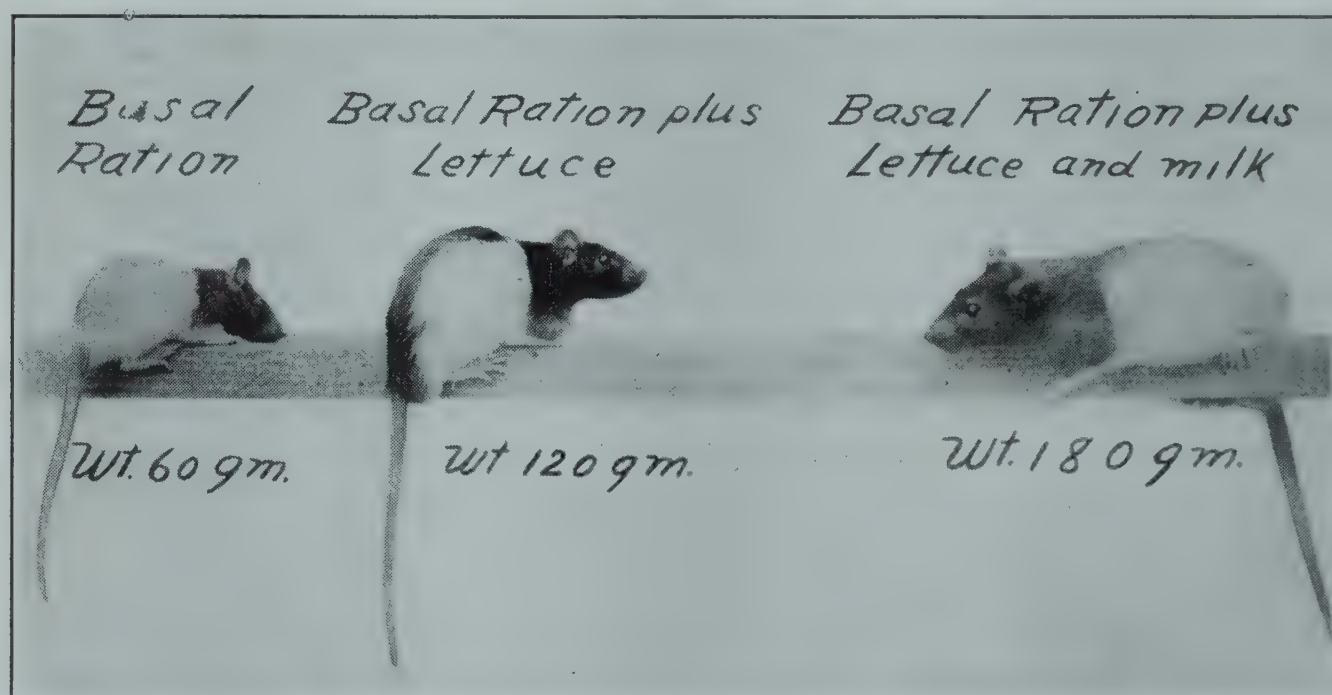


FIG. 18. These rats were of the same age and received the same basal diet with the exception of the supplements noted. This experiment emphasizes the importance of milk and leafy foods in the diet.

International Units for adults. One International Unit (I.U.) is equivalent to the potency of 0.6 microgram (0.6 gamma) of pure β -carotene. For pregnancy and lactation from 6000 to 8000 I.U. are recommended. It must be remembered, however, that these levels are far in excess of actual minimal requirements. Daily allowances for beef cattle range from 25 milligrams (41,666 I.U.) to 55 milligrams (91,666 I.U.), depending on body weight, and daily carotene allowances for swine vary from 2 milligrams to 40 milligrams. Poultry feeds should contain from 2000 to 3300 I.U. of vitamin A per pound of feed, depending upon age, type, and physiological activity of the birds. Breeding turkeys should receive about 4000 I.U. per pound of feed.

Distribution. Plants do not contain vitamin A. Foods of plant origin contain carotene which can be transformed into vitamin A in the animal body. Foods that are particularly good sources of carotene are carrots, sweet potatoes, outer leaves of lettuce and cabbage, apricots, oranges, peaches, tomatoes, alfalfa leaf meal, and pumpkin. Yellow corn is the only cereal grain that makes significant contributions of carotene to the diet.

Foods that are rich in vitamin A include milk, butter, and fat-containing dairy products, egg yolk, liver, glandular organs, and fish-liver oils. Many foods, including the oleomargarines, are now supplemented with vitamin A concentrates.

Vitamin D

Vitamin D is known as the *antirachitic factor* because it prevents rachitis (rickets). Although the disease has been known for centuries, it was not until comparatively recent years that rickets became recognized as a vitamin-deficiency disease.

Rickets, which is primarily a disease of children, is seldom fatal, but it may leave victims with skeletal malformations. Other animal species, such as swine, birds, rats, dogs, lions, tigers, and cattle are also susceptible to rickets.

Vitamin D deficiency symptoms. Rickets is characterized by insufficient deposition of calcium and phosphorus in the bony structures of the body. When infants are afflicted with the disease, the fontanel in the skull fails to close, ribs are usually

beaded, muscles tend to become weak and flabby, and babies are usually restless and fretful. Head sweating is also a characteristic symptom. Older children are often bowlegged, pot-bellied, and susceptible to colds and infections. Muscular impairment and poor tooth development are also characteristic of this disease. Bones of rachitic animals or humans are soft and cartilaginous, which accounts for skeletal malformations.

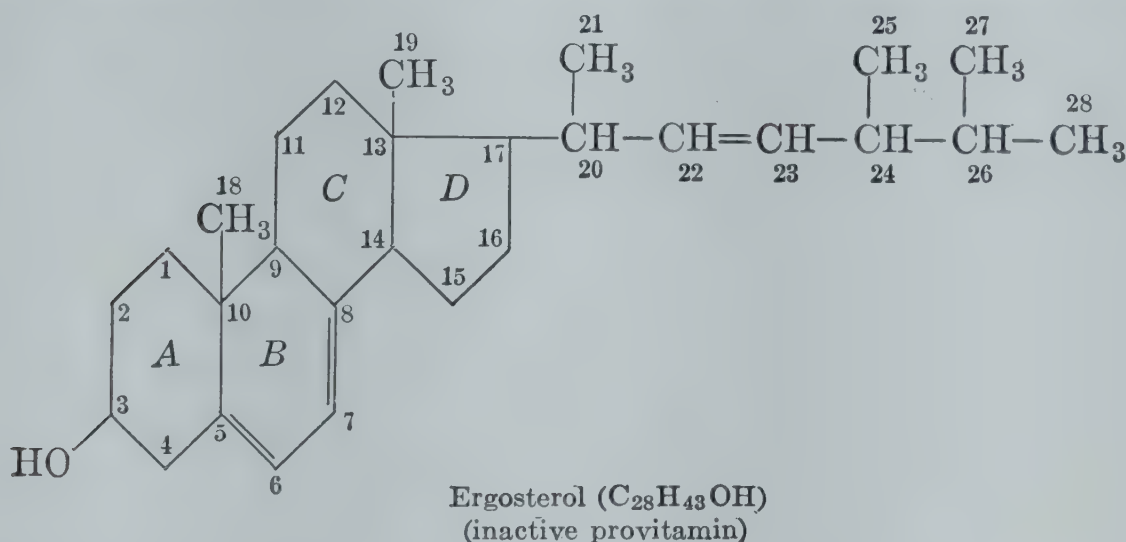
Etiology of rickets. The primary cause of rickets is lack of normal calcification. This abnormal situation may be brought about by diets deficient in calcium or phosphorus or vitamin D or combinations of these. Since calcium and phosphorus are deposited in bones and teeth largely as $\text{Ca}_3(\text{PO}_4)_2$, it is not difficult to understand why normal calcification cannot take place if these important dietary essentials are not present in proper amounts or in proper ratio, one to the other. Experimental rickets can be produced by feeding high-calcium low-phosphorus or high-phosphorus low-calcium rations. If vitamin D is present, calcification is improved, even when the calcium-phosphorus ratio or level is abnormal. In other words, vitamin D seems to function by increasing the efficiency of calcium and phosphorus utilization.

Low-calcium rickets may be accompanied by involuntary muscular contractions (low-calcium tetany). The tetany can be relieved, chemically, by injections of calcium salts. Posterior paralysis of swine is caused by low-calcium diets, resulting in poor vertebral calcification and weakening of muscles. As a result the weight of the body often causes the lumbar vertebra to "slip" and "pinch off" nerves that control the hindquarters.

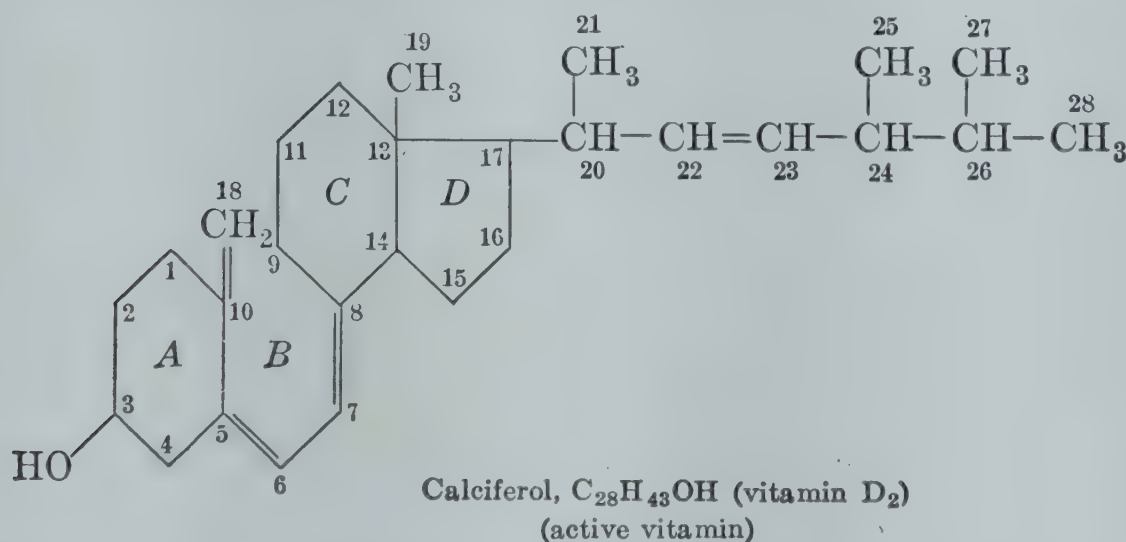
Chemistry. Ten or more chemical compounds have been described that possess vitamin D activity in the prevention and cure of experimental rickets. Two compounds are of practical importance and are commercially available for the use of physicians, veterinarians, and food manufacturers. The natural compound, found in fish-liver oils, is known as vitamin D_3 . Vitamin D_3 is also manufactured commercially by irradiating 7-dehydrocholesterol with ultraviolet light. Another compound, vitamin D_2 , is manufactured commercially by irradiating ergosterol with ultraviolet light. The old term "vitamin D" is still used to refer to the antirachitic potency of foods, regardless

of the specific type of vitamin D that may be responsible for the antirachitic effect.

Ergosterol is a plant sterol obtained from yeasts and fungi. In its natural state, ergosterol has no physiological activity. Cholesterol is an animal sterol found in the unsaponifiable fraction of animal fats and oils. Both types of sterols are derivatives of cyclopentanoperhydrophenanthrene. When ergosterol is activated by ultraviolet light or by bombardment with low-velocity electrons, ring *B* opens, between carbons 9 and 10. The resulting compound is a highly potent antirachitic substance known as *calciferol*, or vitamin D₂. The structure represented by rings *A*, *B*, *C*, and *D* is the cyclopentanoperhydrophenanthrene nucleus.

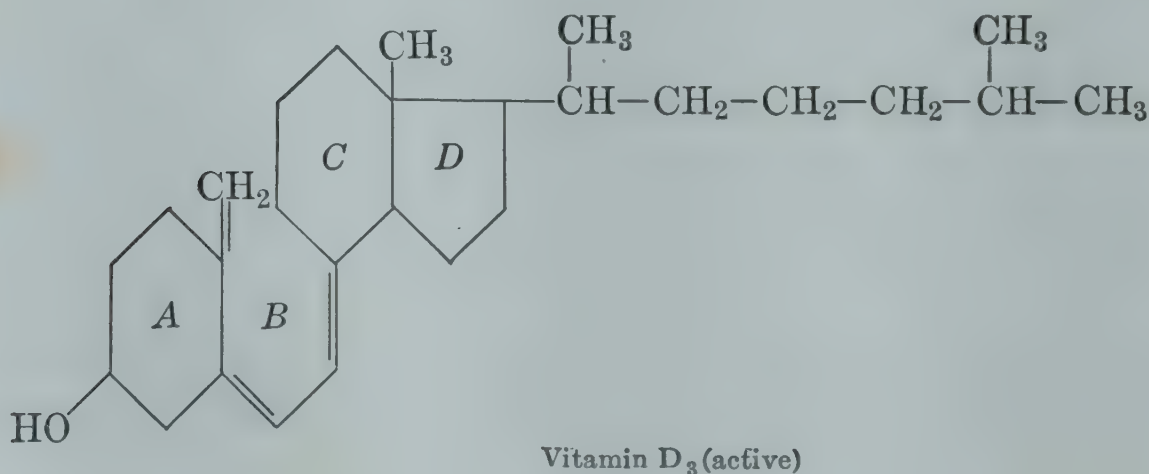
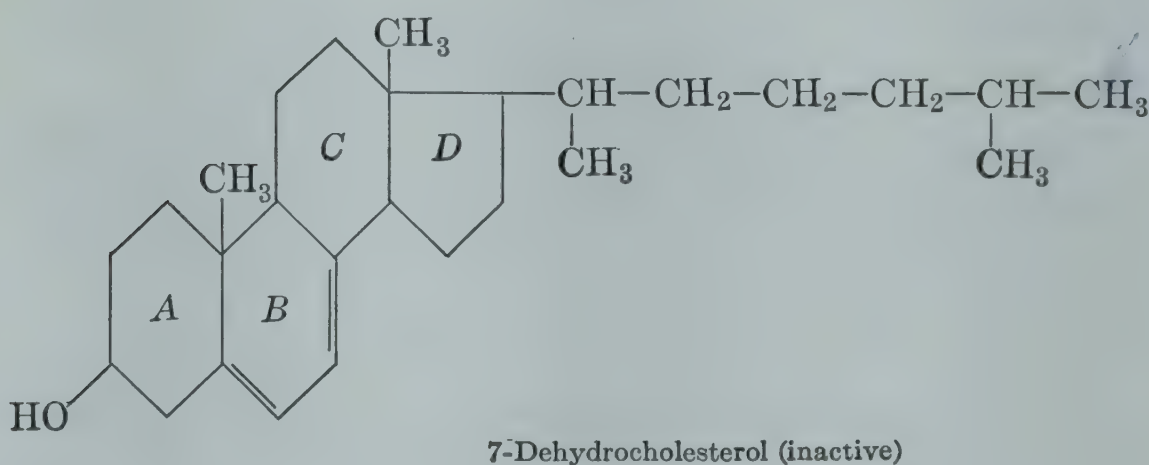
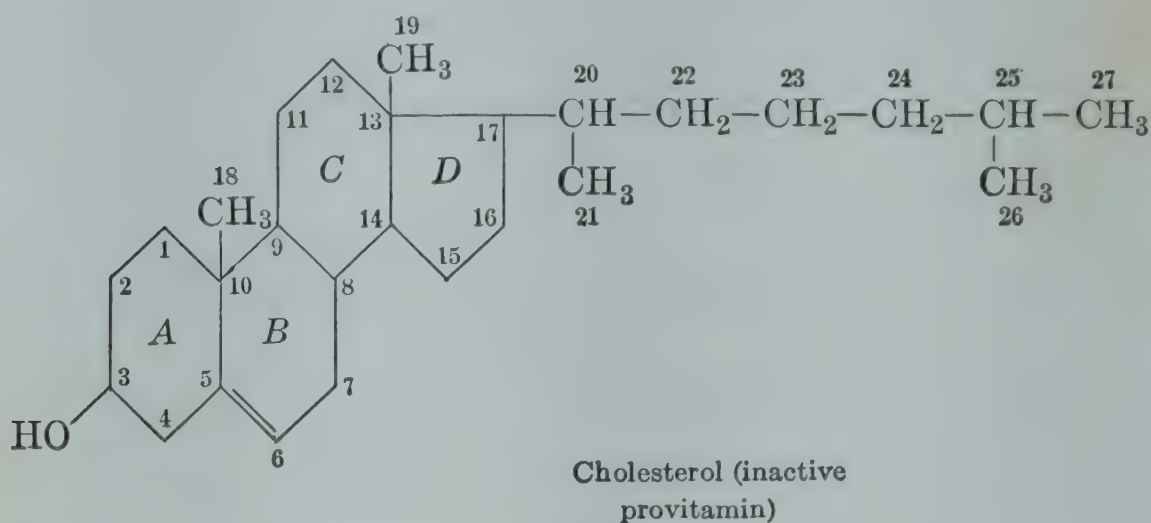


It will be noted that the methyl group (carbon 18) is changed by irradiation to a methylene radical when ring *B* opens at carbons 9 and 10, thus forming vitamin D₂.



Cholesterol, is very similar to ergosterol in chemical structure. It contains the same type of ring structure except that no double

bond exists between carbons 7 and 8 (ring *B*) since these carbon atoms are saturated with hydrogen. The side chain attached to carbon 17 (ring *D*) is saturated and contains no methyl group on carbon 24. 7-Dehydrocholesterol is made from cholesterol by creating a double bond between carbons 7 and 8 (ring *B*) by removing hydrogen. When 7-dehydrocholesterol is treated with ultraviolet light, ring *B* is broken at carbons 9 and 10, with the formation of a methylene radical (carbon 18). The resulting compound is vitamin D₃, which is chemically identical with the natural vitamin D found in fish-liver oils.



Action of vitamin D. When animals are deprived of vitamin D, bones cease to calcify normally. Histological examination shows that cartilage cells multiply but bone-forming cells (osteoblasts) do not form. Previously calcified tissue tends to decalcify and soften, and osteoid tissue fails to calcify.

When vitamin D is administered to a rachitic animal, osteoblasts start to form and osteoid tissue attempts to resume calcification. No completely satisfactory explanation has been advanced to explain the mechanism of vitamin D action. Some workers believe that the primary effect of vitamin D is to increase intestinal absorption of calcium. Other investigators believe that the vitamin affects calcification at the site of bone formation.

Excessive doses of vitamin D are extremely toxic and, at very high levels of intake, actually cause decalcification of normal bony tissues and occasionally cause abnormal calcium deposition in certain soft fleshy tissues. Since vitamin D is essential for normal calcification, most dental authorities stress the necessity of adequate calcium and vitamin D intakes for proper tooth development in children. In order to ensure good skeletal development, breeders of livestock and poultry pay careful attention to the calcium and vitamin D content of growing and breeding rations.

Although human beings and other mammals seem to utilize vitamins D₂ and D₃ with about equal efficiency, this is not true for poultry. For reasons as yet not satisfactorily explained, birds are not able to utilize calciferol (D₂). Consequently all vitamin D preparations manufactured for poultry feeding consist of fish-liver oils, fish-liver oil concentrates, or irradiated 7-dehydrocholesterol, all of which contain vitamin D₃.

Vitamin D unit. The International Unit (I.U.) of vitamin D is equivalent to 0.025 microgram of pure crystalline calciferol which is prepared according to definite specifications. Reference cod-liver oil is then standardized (biologically) against the international standard. The reference samples of cod-liver oil are distributed to laboratories over the world. One (rat) I.U. of vitamin D is equivalent to 1 U.S.P. (United States Pharmacopoeia) unit. Likewise 1 (rat) I.U. is equivalent to 1 A.O.A.C. (Association of Official Agricultural Chemists) chick unit.

Potency of pure D₂ or D₃ preparations approximates 40,000 I.U. per milligram or 40,000,000 I.U. per gram.

Requirements. The recommended daily allowances of vitamin D for infants and young children is 400 I.U.; for special cases, as much as 800 I.U. per day may be prescribed. In active rickets daily doses may vary from 500 to 1500 I.U.

The recommended allowances for poultry are expressed in terms of A.O.A.C. chick units per pound of feed. These range from 180 units for growing chicks to 450 units for laying hens and breeding stock. The allowance, per pound of feed, for turkey poults and breeders is 800 A.O.A.C. units. Small dogs require about 25 to 30 I.U. of vitamin D (daily), and the requirement for large dogs is approximately ten times higher. Daily vitamin D allowances for swine range from 135 to 625 I.U. Cattle require about 300 I.U. per 100 pounds of body weight.

Distribution. The best natural sources of vitamin D₃ are the fish-liver oils. Body oils of fish also contain appreciable amounts of the vitamin. Body oils of salmon and herring are particularly good sources of D. Eggs and butter are relatively poor sources, and milk is so poor in this factor that many states permit the enrichment of milk with vitamin D concentrates. The American Medical Association has recommended that milk, thus fortified, shall contain 400 I.U. of vitamin D per quart.

Vitamin E—the antisterility vitamin

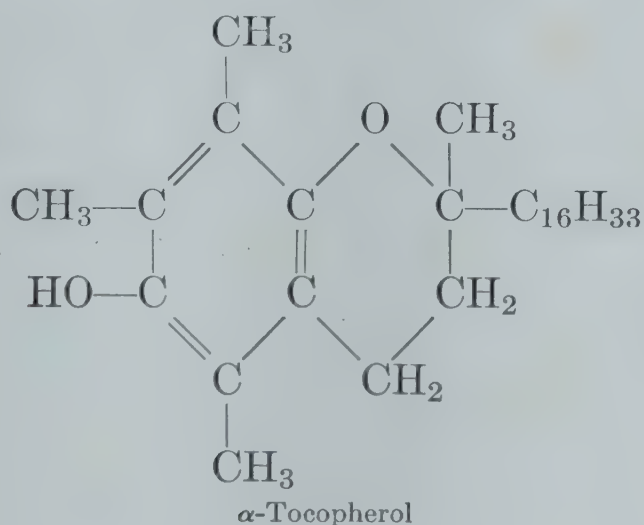
Work leading to the discovery of fat-soluble vitamin E was first initiated in the years 1919 to 1923 when Sure of Arkansas and Evans and Bishop of California discovered that certain types of diets bring about a type of sterility in rats which can be cured and prevented by feeding natural foodstuffs.

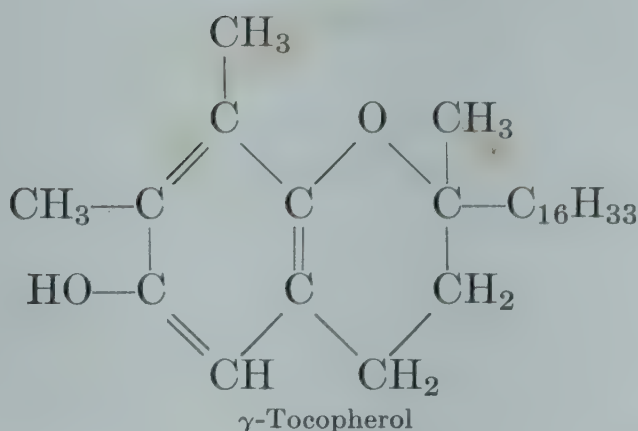
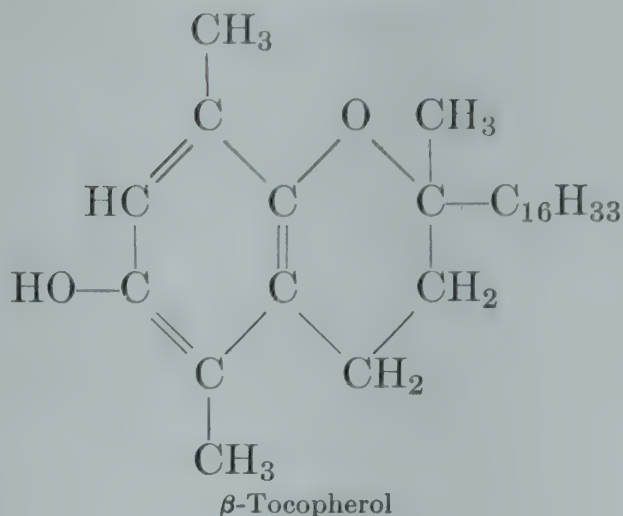
Evans and associates proved that the antisterility factor could be concentrated in fatty extracts of the curative foods. These authors postulated the existence of an unknown factor for which they suggested the tentative name "vitamin X." Eventually Sure suggested that the new fertility factor or antisterility factor be called *vitamin E*.

Subsequent research led to the discovery that female rats could be made alternately sterile and fertile by feeding vitamin E-free

diets, followed by the addition of vitamin E-rich wheat-germ oil to the vitamin E-deficient ration. Vaginal smear techniques were developed to determine conditions of sterility and fertility, without waiting for the young to be born. When male rats are forced to subsist on vitamin E-deficient diets, testes degenerate and the animals become permanently sterile. Female rats, on the other hand, are not permanently injured by vitamin E deprivation. Ovulation and fertilization are not affected, and fetuses start to develop. However, vitamin E-deficient female rats rarely come to term, fetuses are resorbed, and sterility continues unless the diet is enriched with vitamin E-rich foods.

Subsequent chemical studies by California workers revealed that wheat-germ oil and other vitamin E-rich materials contain three different complex alcohols now known as α -, β -, and γ -tocopherols. Later work at the Universities of Harvard, Minnesota, and Zurich, and at the Merck and Company laboratories in this country led to the proof for structure of these compounds. Although they have been synthesized in the laboratory, commercial synthesis is not economically feasible. They are being prepared commercially from soybean oil by molecular distillation. Chemically the tocopherols are substituted derivatives of the chromane type containing a 16-carbon (phytyl) side chain and a hydroxyl group on the benzene ring. They vary in the number or position of substituent methyl groups. α -Tocopherol has the empirical formula $C_{29}H_{50}O_2$ and contains four methyl groups. β - and γ -Tocopherol have the empirical formula $C_{28}H_{48}O_2$, contain but three methyl groups, and differ only in the position of one methyl group. A fourth tocopherol (δ -tocopherol) has been identified, which contains but one methyl group on the aromatic ring.





From the standpoint of antisterility effects on rats, α -tocopherol possesses the greatest potency. All tocopherols act as antioxidants or inhibitors of oxidation. For this reason they have enjoyed limited use by food manufacturers to prevent off flavors and oxidative spoilage in processed foods.

Although some workers have advocated the use of the tocopherols in the treatment of sterility in domestic animals and humans, most authorities are in disagreement regarding the efficiency of the results obtained.

Muscular dystrophy (degeneration of striated muscle), produced in guinea pigs and rabbits by feeding tocopherol-deficient diets, can be prevented and cured by tocopherol therapy. "Stiff lamb disease," which is caused by muscular dystrophy, is said to respond to tocopherol feeding. No satisfactory theory has been advanced regarding the function of tocopherols in the body cells and tissues. Work by Harris and associates indicates that feeding of tocopherols lowers the animal requirement for vitamin A, presumably by preventing oxidative destruction of vitamin A in the digestive tract or in the tissues. No requirements have been established for vitamin E, although suggested allowances

for different types of animals have been advocated by a few workers.

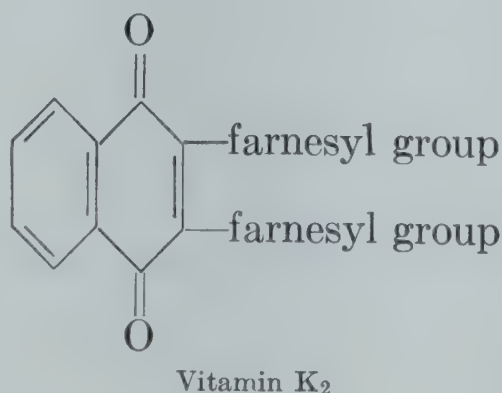
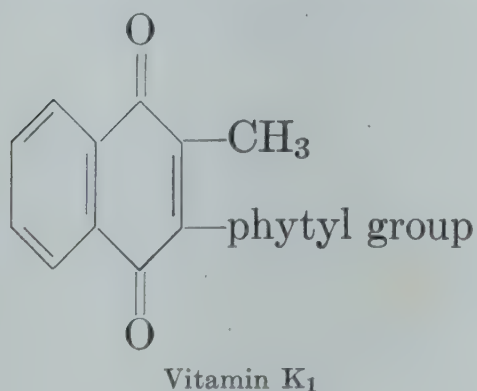
Vegetable oils, such as wheat-germ oil, cottonseed oil, and soybean oil, are rich sources of vitamin E. Olive oil does not contain the vitamin. Small amounts of vitamin E are found in leafy plants, meat, milk, eggs, and some fruits.

Vitamin K

This type of fat-soluble vitamin is known as the anti-hemorrhagic vitamin because it promotes blood coagulation. It was first recognized by Dam of Copenhagen in 1929. By means of artificial diets Dam was able to produce subcutaneous and intramuscular hemorrhages in chicks. These hemorrhages could be prevented and cured by feeding the unsaponifiable fraction of hog-liver fat or alfalfa-leaf oil. In 1934 Dam postulated the existence of a fat-soluble factor necessary for blood coagulation.

In 1935 Dam was able to prove that the antihemorrhagic factor plays a role in the formation of prothrombin, a zymogen necessary for normal blood coagulation. A deficiency of vitamin K leads to a lowering of the prothrombin content of blood. This is known as *hypoprothrombinemia* and results in a prolongation of clotting time of blood which is evidenced by persistent hemorrhages. Clotting time of blood is used routinely as a measurement of vitamin K deficiency.

Chemical investigations led to the isolation and identification of two factors, vitamins K₁ and K₂. Vitamin K₁ was isolated from alfalfa-leaf oil, and vitamin K₂ was prepared from putrefied fish. Both substances are naphthoquinone derivatives. Vitamin K₁ is 2-methyl-3-phytyl-1,4-naphthoquinone, and vitamin K₂ is 2,3-difarnesyl-1,4-naphthoquinone.



A synthetic compound, 2-methyl-1,4-naphthoquinone, is more potent than the natural vitamins K₁ and K₂. This compound is sold for clinical purposes under the name menadione and is used as a standard for the measurement of vitamin K activity.

Vitamin K can be absorbed and utilized only in the presence of bile salts. Consequently any disease or injury that obstructs the normal flow of bile from the liver tends to bring about vitamin K deficiency, lowered prothrombin formation, and persistent bleeding. An example of such a disease is obstructive jaundice. Under such conditions vitamin K must be administered with bile salts. Water-soluble analogs of vitamin K have been synthesized which can be injected, thereby eliminating the necessity for the administration of bile salts. Hemophilia, a "disease of royalty," is not caused by lack of prothrombin. Consequently "bleeders" of that type do not respond to vitamin K therapy.

Under normal conditions all animals can obtain sufficient vitamin K in the food and by intestinal synthesis. The dose of menadione should not exceed 2 milligrams, and treatment should be limited to 28 days because prolonged treatment causes toxic symptoms.

Vitamin K and dicoumarol are antagonistic to each other. The former enhances prothrombin formation, whereas the latter has the opposite effect.

Natural sources of vitamin K include alfalfa, kale, spinach, tomatoes, soybean oil, putrefied fish, and bran.

VITAMIN ASSAY METHODS

It is impossible to discuss in detail all methods for determining each of the known vitamins. Most methods of assaying food materials can be classified under the following headings:

1. Biological methods.
2. Chemical methods.
3. Physical methods.
4. Microbiological methods.

Biological methods

As the name implies, biological methods depend on the use of experimental animals to determine the presence or absence of a specific vitamin in a food mixture. Animals most commonly used for vitamin assays are rats, mice, pigeons, and chickens. Guinea pigs are used almost exclusively for biological estimations of ascorbic acid (vitamin C). Hamsters have been used for certain types of vitamin assays, and higher animals, such as the dog, have been used to a limited extent.

In general the principles involved in biological assays are similar. Control groups of animals are fed a so-called chemically purified ration consisting of all known nutrients (protein, fat, carbohydrate, mineral salts, and vitamins) necessary for normal health and growth. This control ration contains optimal amounts of all vitamins, including the vitamin to be tested.

Similar groups of experimental animals receive the same ration as that fed to the control animals, except that the vitamin to be tested is omitted. As a result the experimental animals develop typical symptoms of vitamin deficiency which can be prevented or cured by adding known amounts of the food containing the vitamin to be assayed. Biological assays are of two general types: (1) prophylactic methods and (2) curative methods.

Prophylactic tests are made by adding small graduated amounts of the food to be tested to the vitamin-deficient ration at the beginning of the feeding tests in order to find the least amount of food necessary to prevent vitamin deficiency symptoms and to produce a rate of growth comparable to that obtained with the control rations which contain known amounts of the vitamin. When the *curative method* is used, several groups of animals receive the vitamin-deficient ration until they begin to lose weight and develop symptoms of vitamin deficiency. At this point control groups receive a sufficient amount of the pure synthetic vitamin to cure the deficiency symptoms and cause the animals to grow at a required rate. Comparable experimental groups of vitamin-deficient animals receive graduated amounts of the food to be tested until an amount is found which

contains sufficient vitamin to bring about growth responses comparable to that obtained with the control animals.

Pure reference standards in the form of chemically pure synthetic vitamins are used for control studies whenever possible. Crystalline thiamine hydrochloride, riboflavin, and L-ascorbic acid are examples of pure reference standards used in assaying foods for vitamins B₁, B₂, and C, respectively. Crystalline β -carotene has been the international standard for vitamin A assays, but it will soon be superseded by crystalline vitamin A acetate. Vitamin D is determined biologically by producing vitamin D deficiency (rickets) in rats and finding the minimum amount of food required to initiate recalcification in the bones. This method involves sacrificing the animals in order that the bones can be examined microscopically. When chickens are used for vitamin D assays, the prophylactic method is followed, and the ash content of the bones is considered the criterion of vitamin D potency. Rations of a special type, containing abnormal calcium-phosphorus ratios, are used for the production of experimental rickets.

Chemical methods

A few vitamins can be determined by chemical methods. These methods have the advantage of conserving time and money, since they are less laborious and time consuming than biological assays. In general, chemical methods are of two types: (1) *colorimetric* methods and (2) *fluorimetric methods*.

Colorimetric methods depend on the formation of stable colored compounds or complexes that can be measured in a colorimeter. Vitamins A and C are vitamins that can be measured by colorimetric methods. Vitamin A forms a blue-colored complex with antimony trichloride in chloroform. When vitamin A is extracted from a food material, after saponification, it can be treated with the antimony trichloride reagent and the color produced can be compared with the color produced by known amounts of pure vitamin A, using the same reagent. The depth of color is proportional to the concentration of vitamin A.

Vitamin C (L-ascorbic acid) can be titrated with a purple dye (2,6-dichlorobenzenone indophenol). The dye is a mild oxidizing

agent which oxidizes L-ascorbic acid to dehydroascorbic acid. In the process the purple dye is reduced to a colorless compound. Thus the dye serves as its own end-point indicator. Pure crystalline ascorbic acid is used as a reference standard, and the vitamin content of the unknown food is determined in terms of the reference standard.

Fluorimetric methods depend on the formation of a fluorescent substance. Thiamine and riboflavin can be measured by this method. When these vitamins are subjected to mild oxidation, they become fluorescent when irradiated with ultraviolet light. The fluorescent molecule has the property of changing invisible short waves of ultraviolet light to visible light. The visible light activates a photoelectric cell, which in turn activates a galvanometer. The galvanometer readings are proportional to the amount of fluorescence, and the fluorescence is proportional to the amount of vitamin present. By using pure thiamine or riboflavin as reference standards, the B₁ or B₂ content of foods can be estimated by fluorimetric methods.

Physical methods

The most common physical method used for estimating vitamins is the spectrographic method. This method depends on the property possessed by chemical substances to absorb light of different wavelengths in varying amounts, depending on the chemical constitution of the molecule. As a result each vitamin has characteristic light absorption properties with absorption maxima at definite wavelengths. It is possible to estimate the amount of vitamin in a given solvent by spectrographic measurements and the determination of extinction coefficients. Vitamins A and D are estimated by this method.

Microbiological methods

These methods involve the use of microorganisms which, by previous tests, have been found to require certain vitamins for growth. Not all microorganisms have the same vitamin requirements. Some microorganisms can synthesize certain vitamins; others cannot. When a microorganism requires a vitamin, it

will not grow normally until that vitamin is added to the nutrient medium. Thus it is possible to use the rate of microbial growth as an index of vitamin potency.

Many types of organisms are used for vitamin assays. Acid-producing organisms have found wide use in vitamin research since the amount of acid produced can be easily measured by titration. To cite a typical example, the acid-producing microorganism *Lactobacillus casei* will not grow normally if the nutrient medium is lacking in folic acid (pteroylglutamic acid). Control tubes containing folic acid-deficient nutrient media are supplemented with graduated amounts of pure folic acid and then inoculated with a pure culture of *L. casei* and incubated for a specified period. The acid produced in each tube is measured by titration with a standard alkali solution. Similar tubes containing graduated amounts of the food to be tested are treated in a similar manner, and the amount of acid produced is titrated. By comparing the acid production in the experimental tubes with the acid production in the control tubes containing pure folic acid, it is possible to estimate the amount of folic acid with speed and accuracy.

Microbiological methods are finding increasing use in the estimation of many other types of compounds, particularly amino acids. A wide variety of microorganisms now being used for this type of work includes acid-producing bacteria, fungi, neurospora, and yeasts.

REFERENCE

ROSENBERG, H. R. *Chemistry and Physiology of the Vitamins*. Interscience Publishers, New York, 1945.

19 · Energy Metabolism

In the preceding chapters we discussed some of the chemical changes that take place in the animal body during metabolism. These chemical changes are accompanied by energy transformations most commonly noted by the production of heat. There are, however, other types of energy transformations. Those chemical changes that are oxidative in nature and are characterized by the evolution of heat are known as *exothermic reactions*. Hydrolyses are characterized by no perceptible heat changes and, for this reason, are known as *isothermic reactions*. When heat is absorbed during chemical changes, as in reduction reactions, the process is said to be *endothermic*.

Animals, unlike plants, receive their food ready-made; hence exothermic reactions predominate in the animal body. The greater part of the chemical energy of foods consumed by animals is eliminated as heat. In herbivorous animals a considerable amount of the gross energy of the feed is eliminated in the feces as undigested material which has not been subjected to oxidative changes in the body.

Owing to the fact that proteins, fats, and carbohydrates play such an important part in energy production, it has been found that energy measurements are very useful in making comparisons of foods and food mixtures in relation to their potential nutritive values. To do this, it is necessary to have some common unit by which the nutritive values of these foods can be measured. This unit is the *large Calorie* (spelled with a capital C) which is equivalent to 1000 calories. A *therm* is equivalent to 1000 Calories. A calorie is defined as the amount of heat required to raise the temperature of 1 gram of water 1° C.

MEASUREMENT OF HEAT OF COMBUSTION

For the measurement of the *gross energy* values of foodstuffs it is necessary to use the bomb calorimeter. There are several modifications of this instrument, but it consists, essentially, of a heavy metal bomb, lined with platinum, gold, or other non-corrosive metal. The bomb contains a platinum receptacle for the weighed sample of food. A heavy metal cover is screwed tightly to the bomb, and oxygen is introduced through suitable openings until the pressure reaches about 300 pounds per square inch. The bomb is immersed in a known weight of water, which is allowed to come to a constant temperature in an adiabatic container which prevents heat losses resulting from conduction or radiation. The food material is "fired" by sending a current of electricity through a very fine iron wire, which is in contact with the foodstuff inside the bomb. By means of a thermometer which records extremely slight changes in temperature, the temperature rise of the water may be measured. Owing to the excess of oxygen in the bomb, combustion is immediate and complete. The heat produced is conducted to the water, and the temperature rise of the water is noted. From data thus obtained it is possible to calculate the total heat (expressed as Calories) released by a known weight of any food material. This is usually referred to as the *gross energy* of the feed.

When pure carbon is burned in a bomb calorimeter it is found that the heat liberated is equivalent to 8.08 Calories per gram of carbon. An equal weight of hydrogen, on the other hand, will yield 34.5 Calories under the same conditions. When hydrocarbons are burned, the amount of heat is practically equivalent to that which would have been obtained if the carbon and hydrogen equivalents had been burned separately. This is not the case, however, when carbon and hydrogen are already combined with oxygen, namely, in the fats and carbohydrates. The reason for this lies in the fact that a portion of the carbon and hydrogen is already oxidized. As a result the total heat obtained in the combustion of carbohydrates and fats is not proportional to the amount of carbon and hydrogen potentially capable of combustion. When proteins are burned, the products

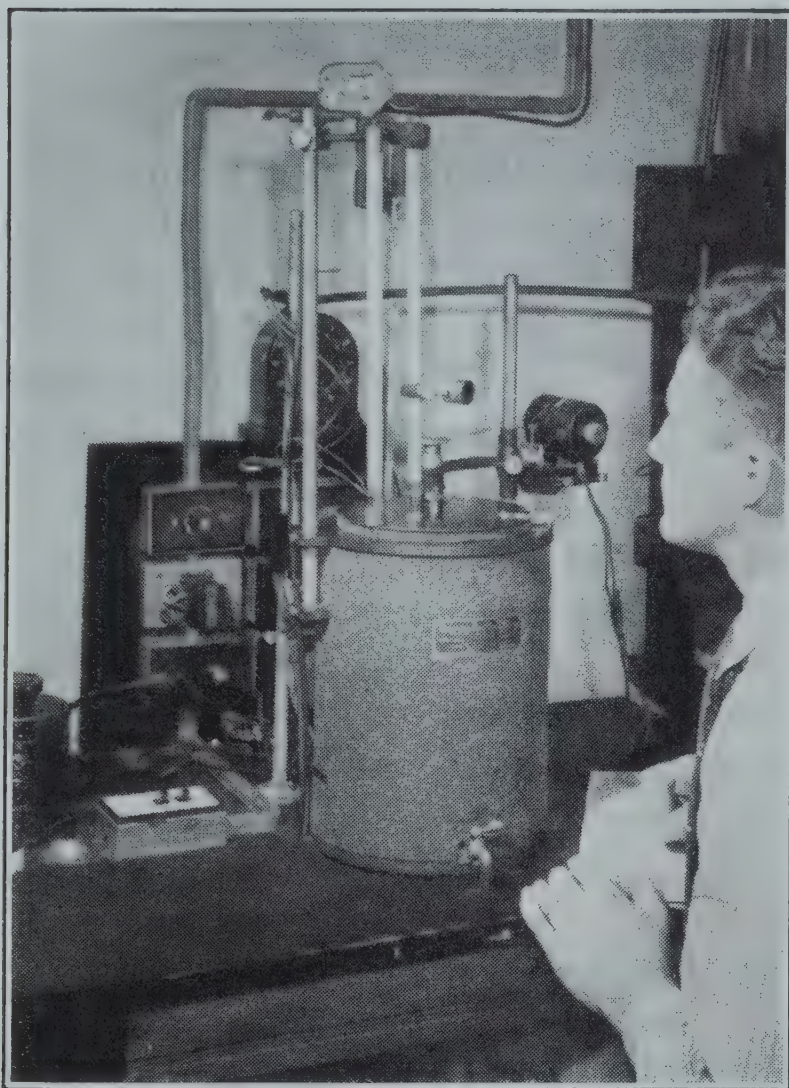


FIG. 19. Measurement of the gross energy of foods by means of the bomb calorimeter. (Courtesy of Dr. R. W. Swift.)

are carbon dioxide, water, and nitrogen gas. The latter does not contribute to the heat of combustion. If sulfur is present, a slight amount of heat is produced in changing the neutral sulfur to sulfur trioxide.

Research work has shown that complete oxidation of these organic food constituents in the bomb calorimeter yields the following (average) values:

Proteins	5.70 Calories per gram
Carbohydrates	4.10 Calories per gram
Fats	9.50 Calories per gram

When these substances are metabolized in the body, however, they are not completely utilized. It is estimated that the following figures represent the average amounts of these constituents absorbed by the animal body:

Proteins	92% absorbed
Carbohydrates	98% absorbed
Fats	95% absorbed

After absorption has taken place, it is found that the body does not burn these compounds completely. Protein, for example, is not completely oxidized, and a portion of its unburned carbon and hydrogen is excreted in the form of urea. This loss of combustible material amounts to about 1.3 Calories per gram of protein burned. Correcting for this loss of unoxidized materials, the following data are obtained:

From proteins (5.65 – 1.30)	4.40 Calories per gram
From carbohydrates	4.10 Calories per gram
From fats	9.50 Calories per gram

Correcting for the loss of unabsorbed or undigested food materials, we obtain the following results:

Proteins	$4.40 \times 0.92 = 4$ Calories per gram
Carbohydrates	$4.10 \times 0.98 = 4$ Calories per gram
Fats	$9.50 \times 0.95 = 9$ Calories per gram

Although these figures are only approximate, they are sufficiently accurate to be used in calculating the total energy value of any feeding material. For example, the average composition of ordinary white bread is as follows:

Water	35.00%
Proteins	9.10%
Carbohydrates	53.30%
Fats	1.60%
Ash	1.00%

To obtain the total available Calories per 100 grams of bread, we are able to make the following calculations:

	GRAMS PER 100 GRAMS OF BREAD		CAL- ORIES PER GRAM		CALORIES PER 100 GRAMS OF BREAD
Protein	9.10	×	4	=	36.40
Carbohydrate	53.30	×	4	=	213.20
Fat	1.60	×	9	=	14.40
<i>Total</i>					<hr/> 264.00

Since there are 453.6 grams in a pound, the total available Calories per pound of bread would be $264.00 \times 4.53 =$ (about)

1196. Similar calculations can be made for various mixtures of foods and feeding materials.

DIGESTIBLE NUTRIENTS

The term *total digestible nutrients* is well known to animal feeders. Although this method of food evaluation is not concerned primarily with energy metabolism, it plays an important part in the eventual interpretation of food values. The estimation of digestible nutrients really amounts to a balance study in which the amounts of chemical nutrients consumed are compared with the amounts of these nutrients which appear in the feces. The amount of each nutrient excreted is subtracted from the amount present in the feed, and the remainder is recorded as *nutrient digested*. The amount of the nutrient digested (expressed in per cent of the nutrient ingested) is known as the *digestion coefficient*. Nutrients for which digestion coefficients are usually determined are crude protein, crude fiber (celluloses), nitrogen-free extract (carbohydrates, other than celluloses) and ether extract (fats). Before a digestion trial can be conducted, it is necessary that the food or feed to be tested be fed in constant amounts to the experimental subject for a preliminary period long enough to make certain that the daily fecal output is representative of the daily food intake. Feces are collected for a predetermined experimental period in which careful records are kept of food consumed as well as of food excreted in the feces.

The following data taken from a typical digestion trial are self-explanatory. The mixed ration consisted of alfalfa and cornmeal.

DIGESTIBILITY OF A MIXED RATION BY A STEER

	Crude Protein	Carbohydrates		
		Crude Fiber	Nitrogen-free Extract	Ether Extract
Grams daily intake in ration	246.7	340.1	1143.9	50.7
Grams daily excreted in feces	71.1	220.3	115.0	17.9
Grams of nutrients digested	175.6	119.8	1028.9	32.8
Digestion coefficient (% digested)	71.2	35.2	89.9	64.7

When the animal feeder wishes to calculate the total digestible nutrients of a given feed or mixture of feeds, he merely multiplies the amount of such nutrient in 100 pounds of ration by its digestion coefficient and then combines these values to obtain the total amount of nutrients available to the animal organism, as indicated in the following table:

CALCULATION OF TOTAL DIGESTIBLE NUTRIENTS

Nutrient	Total Nutrients in 100 Pounds, lb	Digestion Coefficients, %	Digestible Nutrients, lb
Crude protein	11.39	71.2	8.11
Crude fiber	15.75	35.2	5.54
Nitrogen-free extract	60.33	89.9	54.24
Ether extract	2.33 ($\times 2.25$)	64.7	3.39
Total digestible nutrients			71.28

The reader will note that the amount of ether extract (crude fat) is multiplied by the factor 2.25, because fat furnishes 2.25 times more energy per unit weight than is furnished by the other nutrients. This procedure places the system, carbohydrates, proteins, and fats, on an energy basis. In actual feeding practice, wide use has been made of *total digestible nutrients* in making comparative evaluations of rations and feed ingredients for domestic livestock. Several factors affect the accuracy of digestion coefficients. Each type of animal varies in its ability to digest certain types of nutrients. Consequently digestion trials must be run on the type of animal on which the results are to be applied. Furthermore the digestibility of one feed component may be affected by the presence of another feed component. The level of feed intake also affects results. In other words, digestion coefficients are of greatest value only when experimental and practical feeding conditions are comparable, so far as type of animal and type of ration are concerned. Evaluation of feeds by this method, however, ignores the losses in methane and urine which may constitute as much as 15 per cent of the gross energy of the ration.

OXYGEN ABSORPTION AND CARBON DIOXIDE PRODUCTION

Another method of evaluating human foods and animal feeds is to express potential food values in terms of Calories. The fundamental principle upon which this is based depends on the fact that foods are burned in the animal organism in much the same way as fuels are burned in a furnace. By means of indirect calorimetry it is possible to obtain information regarding the caloric or heat values of foods burned in the animal body. Fundamentally, all methods of indirect calorimetry depend on the quantitative relationships existing between the amount of oxygen consumed by the person or animal and the carbon dioxide produced when foods are burned during metabolism. In this type of study urinary nitrogen is used as an index of the protein catabolized. If the amount of oxygen consumed (or the amount of carbon dioxide produced) is known, it is possible to calculate the food value of the ration in terms of heat production.

Oxidation of starch. When 1 gram of starch is burned in a bomb calorimeter, 4.20 Calories are evolved. One gram of glucose, under the same conditions, produces 3.74 Calories. The following equation illustrates the relationship between the O_2 consumed and the CO_2 produced when starch is oxidized.



$$162 \text{ grams} + 192 \text{ grams} = 264 \text{ grams} + 90 \text{ grams} + 680.4 \text{ Calories}$$

The *respiratory quotient* is obtained by dividing the volume of CO_2 produced by the volume of O_2 consumed. Grams of O_2 can be converted to liters of O_2 by multiplying by the factor 0.6998; the factor 0.5094 is used to convert grams of CO_2 to liters (at standard conditions, namely, 760 millimeters and $0^\circ C$). In the above equation it will be found that 134.4 liters of O_2 are needed to produce 134.4 liters of CO_2 when starch is burned to produce 680.4 Calories of heat. It can be seen, therefore, that $CO_2/O_2 = 134.4/134.4 = 1.00$. Consequently the *respiratory quotient* for starch is said to be 1.00. It should be noticed, also, that $680.4 \text{ Calories} \div 134.4 \text{ liters} = 5.060 \text{ Calories per liter}$. This means that, for each liter of O_2 consumed (or CO_2 pro-

duced), 5.060 Calories of heat are produced. Glucose, which also has a respiratory quotient of 1.00, gives a caloric value of O_2 equal to 5.007 Calories per liter.

Since there are many types of carbohydrates, it is customary in practical work to use 4.1 Calories per gram and 5.047 Calories per liter of O_2 absorbed as workable average figures for different types of carbohydrates. If the animal organism is burning only carbohydrates, it is possible to determine heat production by multiplying the liters of O_2 consumed by the factor 5.047. Since this can be done without actually measuring the heat produced, it is called *indirect calorimetry*.

Oxidation of fat. When fats are burned in a bomb calorimeter, much more heat is evolved per unit weight of material than in equal weights of carbohydrates, for the reason that fats are richer in oxidizable carbon and hydrogen. In glucose ($C_6H_{12}O_6$) it will be noted that hydrogen and oxygen are present in the same proportion as they exist in water, leaving only carbon available for oxidation. A typical glyceride, tripalmitin ($C_{51}H_{98}O_6$), on the other hand, is not only rich in oxidizable carbon, but it also contains a large amount of oxidizable hydrogen. Consequently fats require more oxygen for complete combustion than do carbohydrates. The following equation emphasizes these facts more graphically:



806 grams + 2320 grams = 2244 grams + 882 grams + 7657 Calories

When calculations similar to those described for carbohydrates are conducted, it is found that 4.716 Calories are produced for each liter of oxygen absorbed, and the respiratory quotient of the tripalmitin is found to be 0.704.

Since the heats of combustion of different glycerides vary slightly, the average figures for caloric value per liter of O_2 consumed and for respiratory quotient are usually given as 4.686 and 0.707, respectively. For the reasons just described, the average caloric value of 1 gram of fat is 9.5 Calories.

Oxidation of protein. Proteins are very complex compounds compared to carbohydrates and fats. When proteins are burned in a bomb calorimeter, all the oxidizable carbon and hydrogen

burn to CO_2 and H_2O , leaving the unoxidized nitrogen as N_2 . However, when protein is oxidized in the animal body, the combustion is much less complete. Some of the nitrogen forms urea ($\text{CO}(\text{NH}_2)_2$) during metabolism, in which form it is excreted in the urine. Consequently the carbon and hydrogen content of excreted urea is lost to the body for heat and energy purposes. Thus it can be seen that urinary nitrogen can be used as an index of protein metabolism.

One gram of average protein has a caloric value of about 5.70 Calories. The amount of potential energy lost by urea formation is about 1.30 Calories. In order to calculate the amount of potential energy available to the animal organism from 1 gram of protein, it is necessary to subtract 1.30 Calories from 5.70 Calories. This yields the figure 4.4 Calories, which is the average amount of potential energy we can expect when 1 gram of protein is oxidized in the animal body.

Thus it should be clear that the nutrition worker can determine whether the body is burning mostly carbohydrates, fats, or proteins, by measuring the O_2 consumed, the CO_2 eliminated, and the amount of urinary nitrogen excreted. This is known as *indirect calorimetry* because heat values are obtained by calculation rather than by direct heat measurements.

In actual experiments it is found that variations in O_2 consumption are small compared with fluctuations in CO_2 production. Consequently it is possible to obtain fairly accurate estimates of total heat production by using only O_2 consumption measurements and by employing an assumed respiratory quotient. Oxygen consumption measurements are preferred to CO_2 measurements in short time experiments, since considerable error may be caused by the irregularity of breathing. Slow breathing results in a temporary storage of CO_2 and an "apparent" low respiratory quotient, whereas involuntary deep or fast breathing results in an "apparent" high respiratory quotient.

In many cases total metabolism is of the greatest interest. Consequently protein metabolism does not need to be taken into account. This is true when the physician conducts a *basal metabolism test* on humans. He merely wishes to know if the total heat production is normal. This is done by measuring

O₂ consumption when the patient is undergoing complete rest. The significance of basal metabolic rate will be discussed later.

Significance of the respiratory quotient. As stated previously the respiratory quotient (R.Q.) is obtained by dividing the volume of CO₂ produced by the volume of O₂ consumed. If the animal body could burn only carbohydrates, R.Q. would be 1.00. If, on the other hand, fats were the only substance being oxidized, R.Q. would be about 0.70. Although it is true that a single type of chemical substance may be making the principal contribution to heat production at a given time, it must be remembered that R.Q. really represents the algebraic sum of all oxidation reactions taking place in the animal organism. Therefore it should be emphasized that it is dangerous and impractical to be too literal in interpreting the significance of the respiratory quotient. Nevertheless R.Q. is a valuable tool for the nutritionist, who works under carefully controlled conditions with healthy normal subjects.

Basal metabolism. This term refers to the metabolism of a relaxed resting subject, 12 to 18 hours after the last ingestion of food. A longer time must elapse after the last food intake when ruminating animals are used as experimental subjects. This is because the nature of the digestive tract of the ruminant is such that heat production is affected for a considerable time after the last food intake. Since basal metabolic rates are affected by size of the person or the animal, by body surface, and by many other factors, it is necessary to establish some type of standard by which basal metabolic rates of people and animals may be compared.

As a result it is customary in evaluating basal metabolism to express the results in Calories per unit time per unit of body surface. The data may be expressed in terms of Calories per hour per square meter of body surface. Body surface of humans may be computed by the height-weight formula of Du Bois; $A = W^{0.425} \times H^{0.725} \times 71.84$, where A is the surface in square centimeters, W the weight in kilograms, and H the height in centimeters.

Practically all modern hospitals are supplied with basal metabolism equipment whereby the basal metabolic rate of patients is determined by measuring oxygen consumption during rest.

Knowledge of the basal metabolic rate is useful in helping the physician diagnose disease. Some diseases impede the basal metabolic rate; others, such as hyperthyroidism, tend to accelerate the metabolic rate.

Various formulas have been proposed for the computation of the surface area of cattle, rats, and other experimental animals. These also depend on the use of an exponential power of the weight and, usually, some other measurement such as height or length. Armsby advocated the formula $S = KW^{\frac{2}{3}}$, where S is the body surface in square centimeters, W is the weight of the animal in grams, and K is a factor or constant for all animals of the same shape. For beef cattle the factor K may vary, depending on the thinness or fatness of the animal. For practical purposes this factor is not far from 10.34, according to Moulton. In addition to size and shape there are many other factors affecting metabolic rate. Among these are muscular tension, temperament, mental state, age, sex, endocrine activity, pathological conditions, and environmental temperatures.

In recent years investigators have tended to use a fractional power of body weight as a direct unit of reference in calculating fasting catabolism of domestic animals. In fact Brody and associates have concluded that the body weight of most animals, in kilos, when raised to the 0.73 power will yield a fairly accurate estimate of the heat production (in Calories) during fasting.

The influence of *size of body* on surface area per unit weight is well illustrated from the following simple example taken from elementary physics. Let us assume that we have two spheres, the diameters of which are 1 inch and 2 inches, respectively.

SPHERE A		SPHERE B
1.00	Diameter (inches)	2.00
3.14	Surface area (square inches)	12.57
0.52	Volume (cubic inches)	4.19
6:1	Surface to volume ratio	3:1

From Armsby's equation, it follows that heat production per unit weight must decrease during growth, since the larger the animal, the less is the body surface per unit weight. Conversely, small animals should lose more heat per unit weight than large

animals, for the reason that the former have relatively more body surface.

Shape also plays a part in the amount of body surface exposed for heat radiation and heat loss. Rose has shown this graphically by the following calculations. Assuming that we have two cylinders, *A*, wide and short, and *B*, tall and thin, but both weighing the same.

<i>A</i>		<i>B</i>
1.00 pound	Weight	1.00 pound
2.60 inches	Diameter	1.30 inches
2.60 inches	Height	12.20 inches
31.80 square inches	Surface area	48.30 square inches

If we wish to make a practical application at this point, we can say that a tall, thin person should have more surface area per unit weight than a fat or "stocky" type of person. This principle also applies to domestic animals.

METHODS OF MEASUREMENT

There are several ways of obtaining experimental data leading to information regarding food or feed values for man and domestic animals. All these methods yield valid and dependable information. Some methods are limited to the use of small experimental animals; others are best suited for measurements on domestic animals or human subjects.

Direct calorimetry

As the title implies, this method involves direct measurement of heat production. Heat may be measured in a number of ways, but the most common is that which involves the rise in the temperature of a known volume of water. Probably the earliest type was the ice calorimeter. This type, used by Lavoisier in his studies, consisted essentially of an inner chamber totally surrounded by crushed ice. A third (outer) chamber also contained crushed ice. The experimental animal was placed in the cold inner chamber, under conditions that cannot be considered ideal for experimental work. The ice in the outer chamber

functioned as a heat buffer for the heat of the surrounding air. The inner ice chamber was constructed with an outlet pipe and stopcock. As the ice melted in the inner chamber, owing to the body temperature of the animal, the water could be collected and measured. Since it was known that 79.24 gram-calories are required to transform 1 gram of ice at 0°C into water at the same temperature, it was possible to calculate the heat output of the animal under these conditions. This type of calorimeter is known as a *latent heat calorimeter*, since it involves a change in the physical state of a calorimetric substance.

Direct calorimetry requires that two main factors be measured: (1) the amount of heat by radiation and conduction and (2) the amount of heat represented by the water evaporated by the experimental subject. The ratio of these amounts under normal conditions is about 3 or 4 to 1.

The heat of radiation and conduction, in the type of direct calorimeter designed by Atwater and Rosa, is removed by a stream of cold water flowing through copper pipes which form a series of coils near the top of the chamber. Armsby and Fries adapted this type of apparatus for experimental work with farm animals. The temperature of the water as it enters and as it leaves the chamber is recorded at 4-minute intervals throughout an experimental period of 3 days. The rise in temperature of the water multiplied by the weight of water represents the amount of heat removed. An important detail in the operation of the apparatus consists of the adjustment of an insulating shield which may be drawn up at the discretion of the operator to insulate the absorber system from the air of the chamber. Heat is produced at a greater rate when the animal is standing than when it is lying, and by means of this regulatory device, which in effect modifies the absorber system capacity, it is possible to remove the heat as it is produced and to maintain the incoming water at a constant temperature and rate of flow. Thus only the temperature of the outgoing water varies with the amount of heat produced. The weight, temperature, and specific heat of any materials introduced into or removed from the chamber are recorded as a basis of calculations in correcting the directly measured heat production. Three walls of the chamber form two dead air spaces containing electric resistance

wires and cold-water pipes which serve, under control of the operator, to maintain adiabatic control of the chamber. By means of thermocouples, very slight differences in temperature between these two spaces may be detected. Electrical resistance thermometers serve to record the temperature of the chamber, which is kept constant throughout an experiment. All electrical readings are made by the use of a Wheatstone bridge and a very sensitive galvanometer. It is possible to feed and water the animal and to remove feces and urine without disturbing the heat balance of the apparatus.

The heat represented by water vapor must be determined and added to the heat of radiation and conduction. Ventilation is provided by a meter pump which draws air from outside through the chamber and discharges it to the outdoors. This pump also measures the total amount of air passing through. Most of the moisture of the incoming air is removed as a result of passing the air over pipes of a refrigeration system. This prevents condensation of moisture in the air flue and respiration chamber, which might occur during humid weather. A continuous aliquot of this air taken from the air flue, just before it enters the chamber, is drawn through weighed glass-stoppered U-tubes which contain proper absorbents for water and for carbon dioxide. Duplicate aliquot samples of the air leaving the chamber are secured in a similar manner. The ratios of the volumes of aliquot samples to that of the total ventilation (all volumes corrected to 760 millimeters and 0° C) together with the weights of water recorded, afford a means of obtaining the amount of water evaporated by the animal. The heat represented by this amount of water vapor (about 0.5 Calories per gram of water) is added to the heat removed by the absorber system. Carbon dioxide and combustible gases are also determined in the incoming and outgoing air, but as these items are not concerned with direct calorimetry they will be discussed later under one of the methods of indirect calorimetry. Verification of the accuracy of the heat and CO₂ measurements is possible by means of an alcohol check test, in which a known amount of pure alcohol is burned in the calorimeter.

Indirect calorimetry

Respiration methods. As the name implies, indirect calorimetry does not involve direct measurement of heat. We have already mentioned the measurement of the basal metabolic rate of hospital patients. This is probably the simplest form of indirect calorimetry, since the method simply involves the measurement of the amount of oxygen consumed by the patient during rest. Oxygen is breathed through a specially devised face mask or mouthpiece connected to the oxygen supply by a tube. Respiration techniques are probably the most important ways of obtaining calorimetric information by indirect methods.

In some cases respiration studies are made with the use of airtight respiration chambers in which the person or animal is forced to remain throughout the experimental period. In early experiments no provision was made to compensate for the depletion of the oxygen supply or for the excessive accumulation of carbon dioxide. Modern respiration chambers are so designed that fresh supplies of oxygen are added as needed. These chambers are of two types, known as *closed-circuit* and *open-circuit* chambers, respectively.

Closed-circuit respiration chamber. This type of apparatus has been largely superseded by open-circuit chambers or by equipment in which a mask or mouthpiece is substituted for the respiration chamber. Fundamentally the old closed-circuit type of respiration chamber consisted of an airtight room in which the person or animal was placed during the test. By means of a blower, air could be circulated from the chamber through a gas absorption train (to remove CO_2 and H_2O) and back into the chamber again. In order to enrich the oxygen-depleted air, oxygen was added from time to time from an oxygen cylinder. Subsequently the air in the chamber was also sampled and analyzed.

Open-circuit respiration chamber. This chamber differs from the closed-circuit type in that fresh air is introduced continuously. Gas meters are installed at points where fresh air enters the chamber and where respired air leaves the apparatus. By

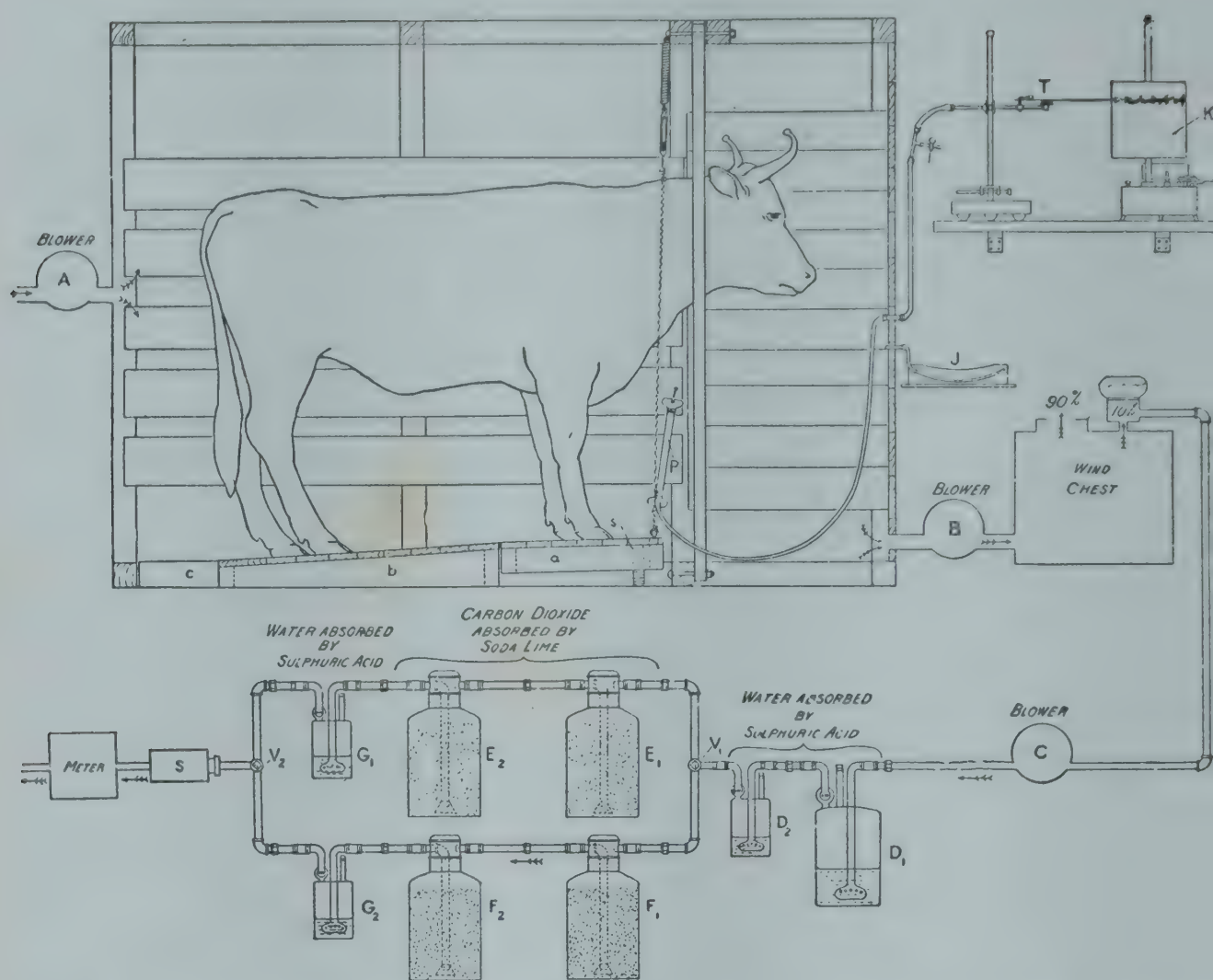


FIG. 20. Indirect calorimetry. Pure outdoor air introduced into chamber by blower *A* is withdrawn by blower *B* and forced into the wind chest. Through one of two circular openings in the wind chest 90 per cent of the air escapes into the room; 10 per cent passes into a sampling can with a rubber bathing-cap top. Blower *C* forces air withdrawn from the sampling can through two sulfuric acids bottles, *D*₁ and *D*₂. Valve *V*₁ deflects air through either one of two sets of soda-lime bottles, *E*₁ and *E*₂, or *F*₁ and *F*₂, and sulfuric acid bottles *G*₁ or *G*₂. Air then passes from the sodium bicarbonate container *S* through meter. A delicate petroleum manometer *J* indicates pressure inside the chamber. To register muscular activity of the animal, the floor is in two sections. At front is a movable platform *a*, supported by two chains attached to springs at top of the chamber and by two compression springs resting on the metal floor of the chamber, only one of which, *s*, is shown. The rear of the floor is a fixed platform *b*, attached at one end to one of the chains supporting the movable platform *a* and at the other end to one of the stall uprights. Changes in tension of air in the pneumograph are transmitted through the rubber tubing with the safety outlet and pinchcock to the tambour *T*, which actuates a small pointer writing on the kymograph drum *K*. (Courtesy of Dr. F. G. Benedict.)

this means the respective volumes of fresh and respired air are recorded. By means of gas absorption equipment incoming fresh air and outgoing respired air are analyzed for CO_2 and H_2O during the experimental period. The energy metabolism may then be calculated from the data obtained. For details regarding the computation of such data, the reader is referred to textbooks on energy metabolism.

Nitrogen and carbon balance method. This method also determines heat production indirectly. Fundamentally this method is based on the determination of the nitrogen, carbon, and energy content of the food and the excreta. The carbon excreted as carbon dioxide (and as methane, when cattle are studied) must also be determined. This is done most advantageously in a respiration chamber. This method does not require the measurement of oxygen consumed. Daily gains or losses of nitrogen are found by subtracting the nitrogen content of feces and urine from the nitrogen content of the food ingested. From the nitrogen balance it is possible to compute equivalent amounts of protein, carbon, and energy, since protein is considered to contain 52.57 per cent of carbon and to yield 5.7 Calories per gram.

The carbon balance may be obtained by subtracting the amounts of carbon contained in the feces, urine, carbon dioxide, and protein gained, from the carbon in the feed. To obtain information regarding storage of fat, the grams of stored carbon are multiplied by 1.307. To convert this to energy stored as fat, the latter figure is multiplied by 9.5 Calories. Finally, heat production is computed by subtracting the energy of the excreta and body gain from the energy intake.

Body balance method. This method does not require the measurement of oxygen consumed or of carbon dioxide produced. This work is usually done with small animals (rats) because litter mate controls are required and all animals must be sacrificed in order that total body tissues can be analyzed. Fundamentally this method depends on a comparison of the composition and energy content of the bodies of control animals with the composition and energy content of carcasses of litter mates that have been fed experimental rations. Food and excreta are analyzed. The amount of nitrogen and energy in the rat bodies

at the start of the experiment is based on the composition of control litter mates killed at the beginning of the experiment. Subsequently similar analyses are made on the litter mates that have undergone the feeding tests. With a knowledge of the energy content of the food consumed and of the excreta, it is possible to compute heat production, which is equal to the



FIG. 21. Metabolism crates for sheep. (Courtesy of Dr. R. W. Swift.)

energy of the food consumed minus the sum of the energy represented by excreta and body gain. Energy data on food, excreta, and body gain are obtained by means of the bomb calorimeter.

METABOLIZABLE ENERGY

We have learned that the total energy of a food is obtained by burning a known weight of the food or feed in a bomb calorimeter. This is known as the *gross energy* of the food. Not all this energy is available to the animal body because food is never utilized completely. Consequently it is desirable to obtain information regarding the proportion of the gross

energy of the food actually *available* for heat production. That proportion of the gross energy of the food actually available for heat production is known as *available energy* or *metabolizable energy*; in other words, the energy actually used for metabolic purposes. Metabolizable energy is obtained by subtracting the energy of the feces, urine, and methane from the energy content of the feed. The gross energy of the protein stored (or lost) must be corrected by taking into consideration the unoxidized portion which appears in the urine.

It is possible to estimate the amount of metabolizable energy required for such vital life processes as normal digestion and blood circulation. When this amount is subtracted from the metabolizable energy, the remainder is known as *net energy*. Net energy of a feed or food is that proportion of the metabolizable energy which is used for maintenance, fattening, growth, and milk or egg production. It can be seen that net energy values will vary, depending upon the use to which the energy is to be put.

Although *metabolizable energy* is preferable to *digestible nutrients* as a measure of nutritive value, it is not the ideal measure for the reason that a portion of it is lost as heat for which the body has no use. It is for these reasons that *net energy* is, theoretically, the most exact measure of nutritive value. However, net energy values vary from one feed to another and are influenced by factors, such as activity, which bear no relation to the nutritive value of the feed. One factor contributing to these variations in heat increment is the *specific dynamic effect* of feed and different combinations of feed ingredients. Unfortunately, therefore, there is a different net energy value for a given food or combination of foods as the plane of nutrition changes.

From the standpoint of practical feeding, *digestible nutrients* are the most practical measure for the calculation of feeding standards, since digestible nutrient tables are now available for most of the foods and feeds commonly fed in this and other countries. It must be borne in mind that such feeding standards are designed primarily as guides. Factors of safety should be allowed to take care of individual variations. Allowances should be made to ensure that the calculated values are higher than the minimum requirements under optimal conditions. Such

"standards" furnish protein and caloric requirements but do not guarantee that mineral and vitamin requirements are met.

As a result of energy studies on human beings and domestic animals it has been possible to establish tables showing the energy allowances or requirements for man and beast under various conditions. Since it is not within the province of this book to discuss practical feeding problems, a few examples should suffice.

As a practical guide in feeding the American people during World War II, the Food and Nutrition Board of the National Research Council established a table of allowances. These allowances do not represent minimal requirements of the individual. These figures include a generous margin of safety in order to ensure maximal nutrition.

DAILY ENERGY ALLOWANCES FOR HUMANS

Men (154 lb)	Calories	Women (123 lb)	Calories
Sedentary	2400	Sedentary	2000
Moderately active	3000	Moderately active	2400
Very active	4500	Very active	3000
		Pregnancy (latter half)	2400
		Lactation	3000
Children up to 12 Years	Calories	Children over 12 Years	Calories
Under 1 year	100 per 2.2 lb	<i>Girls</i>	
1-3 years (29 lb)	1200	13-15 years (108 lb)	2600
4-6 years (42 lb)	1600	16-20 years (119 lb)	2400
7-9 years (55 lb)	2000	<i>Boys</i>	
10-12 years (75 lb)	2500	13-15 years (103 lb)	3200
		16-20 years (141 lb)	3800

As stated previously the most practical method of expressing the energy requirements of domestic livestock is to calculate feeding values in terms of total digestible nutrients.

The Committee on Animal Nutrition of the National Research Council has recommended allowances for various types of domestic animals, a few examples of which are included in the following tables.

DAILY ENERGY ALLOWANCES FOR SWINE

Class	Live Weight, lb	Total Digestible Nutrients, lb
Growing, fattening pigs	50	2.0
	100	3.8
	150	5.0
	200	5.6
	250	6.2
Pregnant gilts and sows and young boars		4.5
Lactating sows and breeding boars		7.5–11.3

DAILY ENERGY ALLOWANCES FOR BEEF CATTLE

Class	Objective	Live Weight, lb	Total Digestible Nutrients, lb
Heifers and steers	Normal growth	600	8.5
Heifers and steers	Normal growth	1000	10.5
Bulls	Moderate activity	600	10.0
Bulls	Moderate activity	1000	12.0
Bulls	Moderate activity	1800	14.0
Yearling cattle	Fattening	600	11.5
Yearling Cattle	Fattening	1000	17.0

DAILY ENERGY ALLOWANCE FOR DAIRY CATTLE

Live Weight, lb	Objective	Total Digestible Nutrients, lb
100	Growth	2.0
600	Growth	8.5
1000	Growth	11.0
1200	Growth	12.0
700	Maintenance	6.0
1000	Maintenance	8.0
1200	Maintenance	9.5
Per 1000 lb (last 6–12 weeks)	Pregnancy	14.0

If the cow is producing milk, more nutrients are required for milk production. Consequently the dairy farmer must supplement the amount of total digestible nutrients required for maintenance and growth, with additional amounts of feed. The amount of total digestible nutrients to be added is based on the pounds

of milk produced and the per cent of butterfat in the milk. A few examples are given in the following table.

ADDITIONAL (DAILY) TOTAL DIGESTIBLE NUTRIENTS REQUIRED
FOR MILK PRODUCTION

Per Cent Fat	T.D.N. per Pound of Milk
3.0	0.28
4.0	0.32
5.0	0.37
6.0	0.42

For detailed information concerning feeding standards for human beings and domestic animals, the reader is referred to standard textbooks on nutrition and animal feeding. (See the Appendix for National Research Council tables of recommended allowances.)

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- Recommended Allowances for Domestic Animals*: I. Poultry; II. Swine; III. Dairy Cattle; IV. Beef Cattle; V. Sheep; VI. Horses. National Research Council, Washington, D. C.
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20 • Carbohydrate Metabolism

The term *metabolism* refers to the chemical changes food substances undergo after they have been absorbed. Some writers use the term *intermediary metabolism* to refer to chemical changes occurring within the tissue cells. The term *anabolism* is sometimes used to refer to synthetic metabolic changes which have to do with the building of tissue. The term *catabolism*, on the other hand, is applied to chemical changes characterized by tissue breakdown. These terms are used largely as a matter of convenience, since it is not possible to draw a sharp line of division, because both types of changes are going on simultaneously and continuously.

So far as carbohydrate metabolism is concerned, we need not give serious consideration to more than three of the simple sugars, namely, glucose, fructose, and galactose. Of these glucose is the most important. The intermediary metabolism of the hexoses is exceedingly complex, and methods for studying the complicated chemical changes are far from satisfactory. In recent years the biochemist has taken advantage of the fact that he can "tag" or "mark" chemical compounds through the use of radioactive or heavy isotopes of carbon and hydrogen. By following these isotopic markers it has been possible to learn a great deal regarding the nature and mechanism of many hitherto unknown changes occurring in intermediary metabolism.

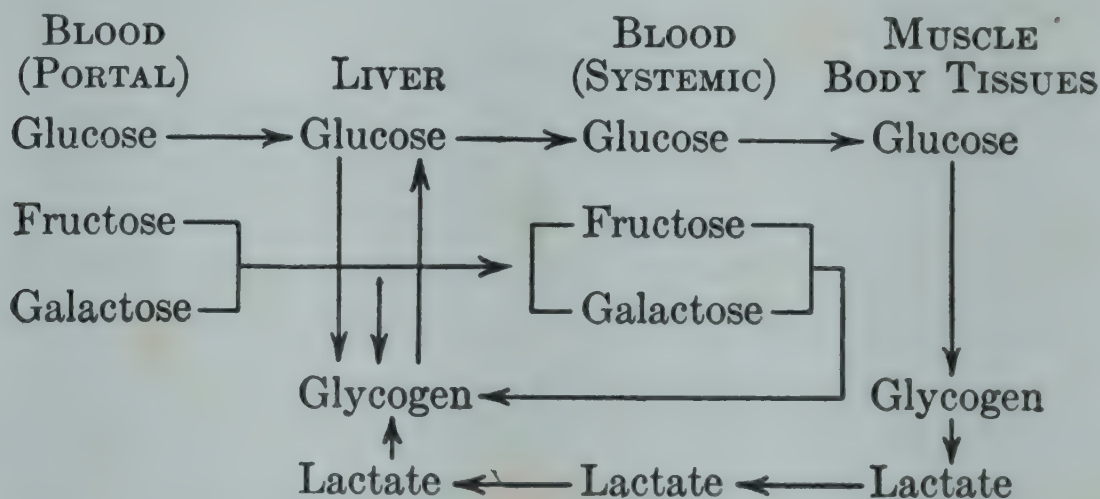
Carbohydrates function primarily as sources of energy, but they also play an important role as constituents of many important biological compounds. In Chapter 16 we learned that, during digestion, digestible carbohydrates are hydrolyzed to monosaccharides before they can be absorbed in the intestine. Of these, glucose, fructose, and galactose are the only ones possessing real physiological importance. During absorption these hexoses react with phosphoric acid, forming phosphate esters. This process is called *phosphorylation*. The rates of

phosphorylation differ, which may explain differences in the rate of absorption of these sugars. Galactose is absorbed more readily than glucose, and glucose is superior to fructose in this respect. The absorbed sugars pass by way of the portal vein to the liver. This organ is able to convert all these hexoses to glycogen, a polysaccharide sometimes known as *animal starch*.

Often the supply of sugar is so great that the liver is unable to convert all of it to glycogen. Consequently some sugar passes into the general blood system, causing an increase in the sugar concentration of the systemic blood. Some of this blood sugar finds its way to the muscle tissues where it is converted into muscle glycogen and stored. It has been calculated that the average human possesses about 200 grams of stored glycogen, which is about equally distributed between liver and muscle tissues.

Glycogen. The glycogen reserve is very important, since it is the source of energy for muscle contraction. During muscle metabolism, glycogen is converted to lactic acid. A part of the lactic acid may be burned, and a portion may be carried to the liver where it is converted to liver glycogen.

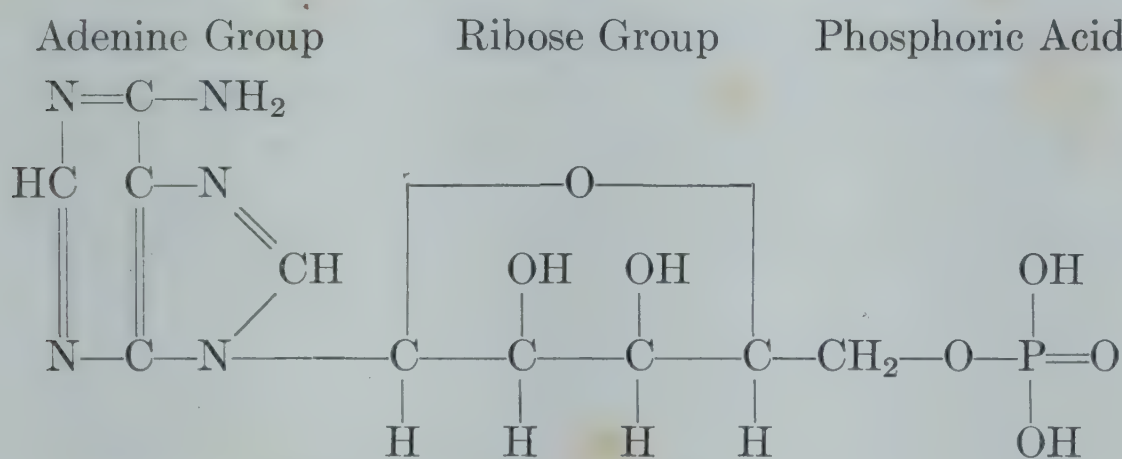
It is interesting to note that, although the liver is capable of forming glycogen from glucose, and glucose from glycogen, muscle tissue is only able to form glycogen from glucose. In other words, in liver the reactions are reversible ($\text{glycogen} \rightleftharpoons \text{glucose}$); in muscle tissue the reaction goes in but one direction ($\text{glucose} \rightarrow \text{glycogen}$). In muscle and other tissues glycogen is oxidized to lactic acid. These relationships are shown in the following diagram:



Glycogen formation is called *glycogenesis*, and hydrolytic glycogen breakdown is called *glycogenolysis*.

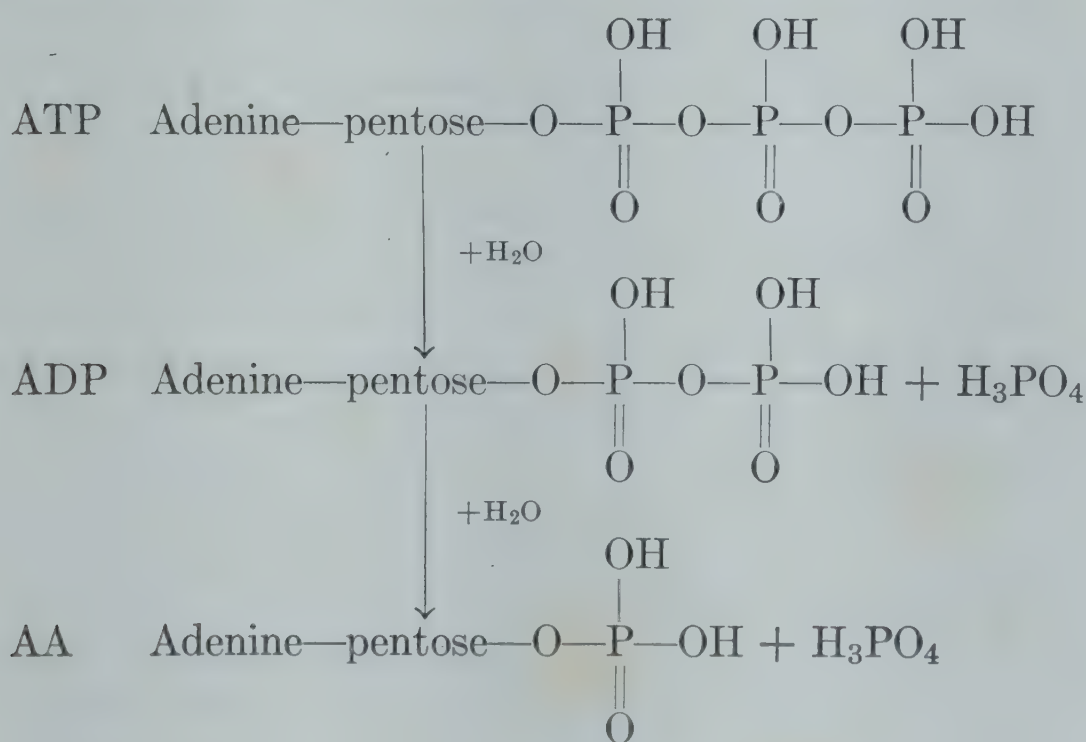
Importance of phosphoric acid. Phosphoric acid plays a dominant role in metabolism. Many reactions depend on phosphate donors and phosphate acceptors. When phosphates combine with organic compounds, the process is known as *phosphorylation*. In order that phosphate groups can react readily with organic compounds, like glucose, phosphate donors must be present in the tissues.

Adenosine triphosphate (ATP) is an important phosphate donor. This compound may be considered as adenylic acid which has been condensed with pyrophosphoric acid. Adenylic acid is a compound consisting of adenine, ribose, and one molecule of phosphoric acid. Adenosine diphosphate (ADP) differs from

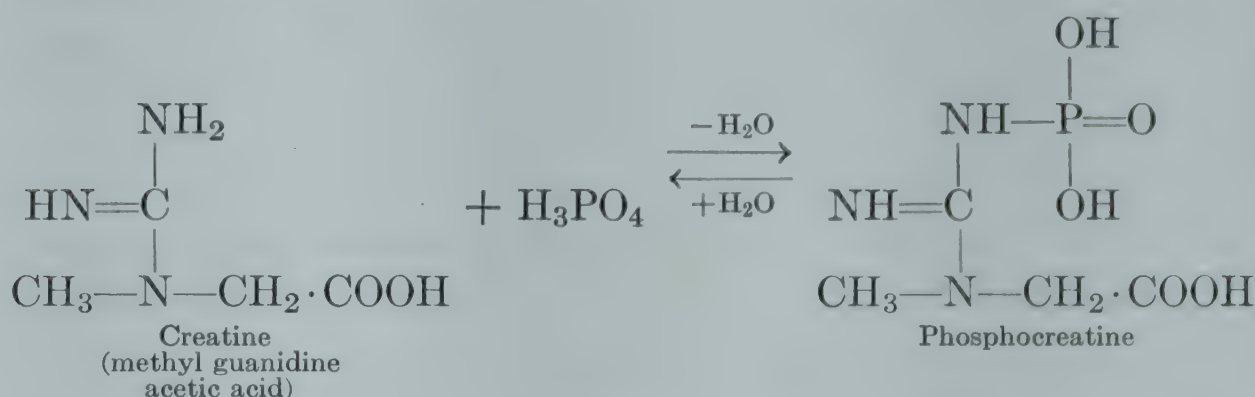


(AA) Adenylic acid or adenine ribose phosphate

adenylic acid by containing two phosphoric acid molecules, and adenosine triphosphate differs from ADP by containing three phosphate groups. These can be compared as follows:



Another phosphate donor is important in muscle metabolism. This compound is known as *creatine phosphate* or phospho-creatine. The importance of these phosphate donors will be discussed later.



Blood sugar. One hundred milliliters of normal venous human blood usually contain from 70 to 130 milligrams of glucose. During sleep the concentration of sugar reaches its lowest level. After meals the concentration of blood sugar rises rapidly, reaches a peak and then gradually falls to the normal level. Some of the factors that control the concentration of glucose in blood are (1) the speed of liver glycogenesis and utilization, (2) utilization of carbohydrate by other body tissues, (3) conversion of carbohydrate to fat, and (4) excretion of glucose in the urine.

When the blood-sugar concentration exceeds the normal level, the condition is known as *hyperglycemia*. Conversely, when the concentration is below normal, the condition is called *hypoglycemia*. When the normal level is exceeded, owing to excessive carbohydrate intake, the term *alimentary hyperglycemia* is applied.

Glycogenesis in the liver. Glycogen formation is accomplished by enzyme systems in the liver cells. Although the hexoses in portal blood play a major role in the formation of liver glycogen, other types of compounds also are important sources of this polysaccharide. When proteins are hydrolyzed and deaminized, the non-nitrogenous fractions form pyruvic acid which can be reduced to lactic acid. In the liver, lactic acid is oxidized to pyruvic acid prior to glycogen synthesis. It has been calculated that more than 50 per cent (by weight) of protein is converted to glucose during metabolism. Glycerol, resulting from fat

hydrolysis, also forms glucose and can be deposited as glycogen. Evidence is lacking to show that appreciable amounts of glycogen are formed from fatty acids.

Glycogenolysis in the liver. In our study of enzymes we learned that phosphorylase enzymes are widely distributed in nature. When glycogen is changed to glucose, it is first changed to glucose-1-phosphate by a phosphorylase enzyme which requires adenylic acid for its action.

When the body calls for increased amounts of glucose, the sympathetic nervous system stimulates the production of an adrenal hormone, which, in turn, stimulates the production of glucose from glycogen. Violent emotions, such as fear and hate, also cause increased concentrations of blood sugar as a result of increased production of adrenalin. On the other hand, insulin tends to counteract the effect of adrenalin by promoting the formation of glycogen from blood sugar.

Muscle glycogenesis. We have learned that liver glycogen can be formed from a number of substances. This is not true of muscle glycogen. Glucose is the only substance muscle tissue can use for glycogen formation. During muscle contraction muscle glycogen reserves are depleted. This calls for more blood sugar which tends to lower the blood sugar concentration. As a result liver glycogenolysis is increased in order that normal blood sugar levels can be maintained.

Muscle glycogenolysis. The first step in muscle glycogenolysis is similar to that of liver, namely, the formation of glucose-1-phosphate. Liver contains a phosphatase capable of hydrolyzing glucose-1-phosphate to glucose. This does not occur in muscle glycogenolysis since muscle tissue contains no phosphatase. As a result muscle glycogen undergoes a complex series of reactions which finally result in the formation of lactic acid. These have been discussed in Chapter 8. This lactic acid can be oxidized to CO_2 and H_2O or transformed into glycogen in the liver. The more important phases of muscle metabolism will be discussed later.

Glucosuria. If the body is functioning normally and if carbohydrate intakes are not excessive, it is impossible to detect glucose in the urine by ordinary methods. However, if the blood sugar rises as a result of excessive carbohydrate intake,

glucose will "spill over" into the urine. This is explained by stating that the *renal threshold* has been reached. This condition is known as *glucosuria*. The kidneys serve as a protective mechanism to prevent hyperglycemia. The renal threshold varies among individuals and may vary from time to time in the same individual. Usually glucosuria occurs when blood sugar levels reach concentrations of 160 to 180 milligrams per 100 milliliters of blood. In the absence of excessive carbohydrate intakes, glucosuria is an indication of diabetes. However, absence of glucosuria is not always proof that carbohydrate metabolism is normal. For example, the renal threshold may be abnormally high, with the result that no sugar can be found in the urine even though the patient is afflicted with hyperglycemia, which is an indication of abnormal carbohydrate metabolism.

Fat from carbohydrate. When the body can no longer store glycogen, excess carbohydrate is stored as fat. Fat is used as a reserve source of energy when and if glycogen supplies are depleted. Much is yet to be learned regarding the mechanism of fat formation from carbohydrates, but it is evident that enzyme systems are involved.

Glucose metabolism in muscle. When muscle contracts, work is done. At first glance it might appear that oxygen is absorbed and glycogen is oxidized with the formation of CO_2 and H_2O . Unfortunately, the reactions are involved and complex. In fact when muscle contracts the reaction is anaerobic. It is only when the muscle relaxes (recovery phase) that oxygen is absorbed and CO_2 is produced. Consequently, the energy of contraction cannot be oxidative in nature.

Recent researches indicate that the energy of muscle contraction is derived from labile compounds containing energy which can be released readily. The most important of these are adenosine triphosphate (ATP), adenosine diphosphate (ADP), and phosphocreatine.

ATP and ADP contain energy-rich phosphate bonds. Hydrolysis of these compounds to simpler phosphate structures is accompanied by a transfer of energy from the energy-rich compound to a receptor substance in the muscle fiber which makes possible the work of muscle contraction. These compounds can

also phosphorylate creatine, glucose, and fructose. When glucose and fructose are phosphorylated, the energy of the energy-rich phosphate radicals is used up in the reaction. However, when creatine is similarly phosphorylated, the resulting phosphocreatine contains an energy-rich phosphate bond which can be used for energy transformations. Details of energy transformations have been discussed in Chapter 8.

So far as carbohydrate metabolism in muscle is concerned, we can summarize the major events as follows: (1) Glycogen forms glucose-1-phosphate. (2) Glucose-1-phosphate combines with more phosphoric acid to form fructose-1,6-diphosphate. (3) Fructose-1,6-diphosphate splits to form 3-phosphoglyceric aldehyde and dihydroxy acetone phosphate, each of which contains but three carbon atoms. (4) 3-Phosphoglyceric aldehyde is thought to be phosphorylated to form 1,3-diphosphoglyceric aldehyde. (5) 1,3-Diphosphoglyceric aldehyde is dehydrogenated to 1,3-diphosphoglyceric acid accompanied by a release of energy. The acid is hydrolyzed to the monophosphate, 3-phosphoglyceric acid. (6) This molecule rearranges to form 2-phosphoglyceric acid followed by (7) loss of H_2O from 2-phosphoglyceric acid, forming the enol form of 2-phosphopyruvic acid which (8) is hydrated to form pyruvic acid and phosphoric acid accompanied by a further release of energy. (9) In the presence of co-enzyme I, pyruvic acid is reduced to lactic acid. It can be seen that lactic acid is the end product of glycogen breakdown in muscle, and the phosphates play an important role in energy transformations.

Thus it may be seen that the lactic acid, which accumulates as a result of muscle activity, may be oxidized, or carried by the blood to the liver and converted to glycogen. The details of lactic acid oxidation are not clear, but many authorities believe that it undergoes oxidation according to the Krebs citric acid cycle (see Chapter 8). This hypothesis postulates the formation of pyruvic acid from lactic acid. The pyruvic acid is thought to combine with oxaloacetic acid to form isocitric acid. As a result of a series of reactions, isocitric acid forms a number of intermediate acids, and the reactions finally terminate in the resynthesis of oxaloacetic acid which can unite with more pyruvic acid to continue the metabolic cycle.

During this series of reactions three molecules of CO_2 are lost. This is equivalent to the oxidation of one molecule of lactic or pyruvic acid. The reader should keep in mind that the energy of these aerobic oxidation reactions is used to rebuild the energy-rich compounds used in the anaerobic phase of muscle contraction.

Abnormal Carbohydrate Metabolism. Beriberi is a dietary deficiency disease caused by a lack of thiamine (vitamin B_1) in the diet. One of the characteristics of this disease is the accumulation of unburned pyruvic acid in nervous tissue and blood. Normal oxidation of pyruvic acid depends upon the presence of thiamine pyrophosphate, a coenzyme known as *coccarboxylase*. Lack of dietary thiamine limits the production of coccarboxylase, thereby preventing the normal oxidation of pyruvic acid. This acid accumulates in the tissues to the point where it acts as a poison.

In diabetes, sugar appears in the urine (glycosuria) because the level of blood sugar (hyperglycemia) is too high or because the renal threshold is too low. When glycosuria is accompanied by hyperglycemia, the disease is called *diabetes mellitus*.

Work by Banting and Best of Canada (1922) led to the discovery that insulin (the pancreatic hormone) controls oxidation of sugars in tissues (see Chapter 17). When certain pancreatic cells (islands of Langerhans) cease to function, insulin is not produced in sufficient amount to maintain normal carbohydrate metabolism. Prior to the discovery of Banting and Best, mortality was high in humans afflicted with diabetes. As a result of these researches, it has been possible to isolate insulin in commercial quantities for the therapeutic treatment of diabetes. Today a person afflicted with this disease has a reasonable chance for a long, active life through the advent of insulin therapy.

Since insulin is a protein, it is destroyed by enzymes in the digestive tract, which makes oral administration inadvisable. Consequently, it must be injected into the muscle tissues. When insulin is combined with zinc and protamine it is absorbed by the tissues more slowly than ordinary insulin, thereby reducing the chances of insulin shock due to overdosage. At the

same time the use of the zinc-protamine-insulin reduces the number of daily injections.

Lack of insulin also hinders normal fat oxidation, causing incomplete oxidation and the formation of toxic products known as acetone bodies. These will be discussed when we consider fat metabolism.

21 • Lipid Metabolism

Representative fatty substances which play an important role in lipid metabolism in the animal organism are fats, phospholipids, and cholesterol. Of these, fats are probably of greatest interest and importance.

During digestion a large part of ingested fats are hydrolyzed to glycerol and fatty acids. In Chapter 16 we learned that the fatty acids may be absorbed as a water-soluble bile salt-fatty acid complex, that some unhydrolyzed fat may be absorbed in the form of finely emulsified fat, and that some of the glycerol is absorbed as free glycerol.

Recent studies have yielded evidence to show that food fats may be incompletely hydrolyzed, yielding monoglycerides, in which but one fatty acid is combined with glycerol. These monoglycerides are absorbed as a bile salt complex. Glycerol and fatty acids are reunited as they pass through the intestinal wall. Some of the fat finds its way to the blood stream via the lacteals and the lymphatic system, while another portion is carried to the liver via the portal vein.

Function of the liver. The liver serves as a temporary storage organ for fat while phosphorylation (phospholipid formation) is taking place. The phospholipid serves as the principal form in which fat is transported to the tissues. Phospholipid formation in the liver can be inhibited by certain poisons or by disease. When this occurs, large amounts of fat may accumulate and be deposited in the liver. Such livers are known as "fatty livers." Certain types of fatty livers respond to choline feeding. Since choline is necessary for the formation of phospholipid (lecithin) from fat, it is to be expected that diets deficient in choline will cause fat deposition in liver. This has been proved experimentally. Choline is said to be a *lipotropic substance*

because of its ability to reduce accumulated fat in the liver.

While we are discussing the function of choline, we should remember that choline also plays an important role in metabolism by serving as a donor of methyl groups to other compounds. This transfer of labile methyl groups is known as *transmethylation*. For example, the indispensable amino acid, methionine, can be synthesized by the methylation of homocystine. Conversely, compounds containing labile methyl groups can furnish methyl groups for the synthesis of choline.

Before we conclude our discussion of the function of the liver in lipid metabolism, it should be pointed out that this organ also functions in the synthesis and esterification of cholesterol. Also, desaturation of fatty acids is thought to take place in the liver.

Fat formed from carbohydrates and proteins. Nearly everyone is aware that carbohydrate-rich diets tend to cause fat deposition and increased body weight. Swine feeders take advantage of this fact in fattening hogs for the market. Overfat persons know that one effective way of reducing body weight is to abstain from eating sweets and starchy foods. Thus it is clear that fats can be synthesized from carbohydrates, in the animal body.

Fats can also be synthesized from proteins. During metabolism, proteins are deaminized. The non-nitrogenous degradation products of protein metabolism may be oxidized or deposited in the tissues as glycogen or fat. Animals that have been forced to subsist on diets consisting almost wholly of protein are able to grow and to deposit appreciable amounts of fat in their tissues.

Food fat and body fat. In general, it may be stated that different types of animals tend to synthesize and deposit tissue fats which are characteristic of the species. However, chemical and physical properties of animal fats can be modified by diet. Hogs, for example, tend to produce a firm type of fat when the ration is rich in carbohydrate. However, if an appreciable amount of carbohydrate in the swine ration is replaced by peanut oil, the tissue fat tends to become soft and oily. This creates practical difficulties in meat-packing plants because the carcasses cannot be handled or piled effectively. Similarly, the chemical and physical properties of butterfat can be modified

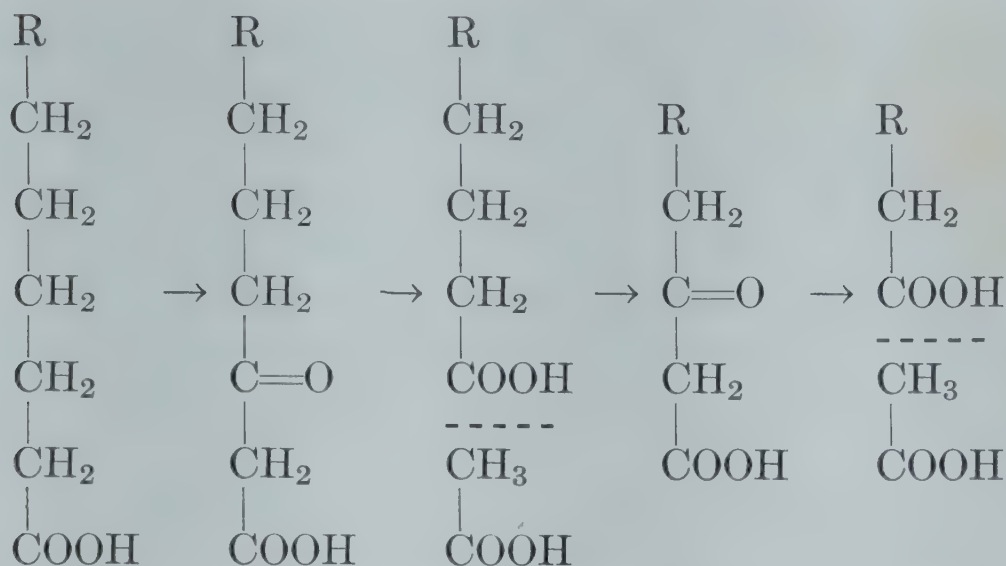
by feeding cottonseed meal to dairy cows. In spite of the fact that dairy cows and other lactating animals possess the ability to synthesize fats, it has been shown that normal synthesis of milk fat cannot take place unless the ration contains some dietary fat. Fat that is stored as an energy reserve is called *depot fat*.

Oxidation of fats. Glycerol resulting from the hydrolysis of fats undergoes oxidative changes similar to those described for carbohydrates, to which it is closely related chemically.

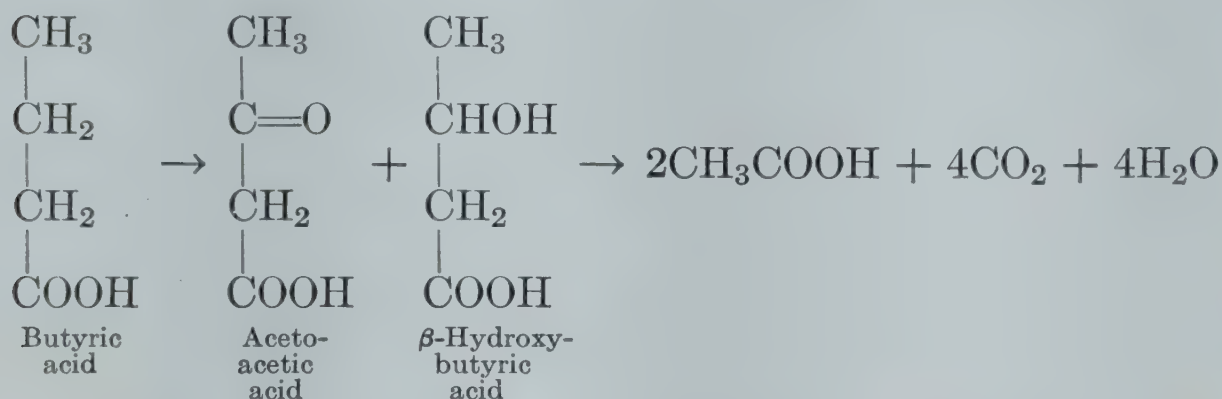
However, the oxidation of fatty acids is a much more complicated process. It was not until comparatively recently that some of the details of fatty acid oxidation have been clarified. The discovery and the use of isotopes have made it possible to follow metabolic changes which could not be studied by the older techniques. In spite of the progress made by the use of isotopic markers, there is still much to be learned regarding fatty acid metabolism. It is evident that all the chemical changes are in a dynamic state of flux. Fatty acid chains are being shortened and lengthened, and saturation, desaturation, and oxidation reactions are occurring simultaneously. The presence of double bonds in an unsaturated fatty acid tends to weaken the carbon chain at the point or points where the double bonds occur.

β Oxidation. One theory of fatty acid oxidation which has received wide acceptance is the β oxidation theory of Knoop of Germany. This worker presented evidence to show that fatty acid chains tend to lose two carbons at a time during oxidation. He postulated that oxidation tends to take place at carbon 3 (β -carbon), thereby forming a keto acid, with a carbonyl radical at the β position. According to this theory, the next step in fatty acid degradation is a cleavage at the (β) carbonyl group, thus forming a new fatty acid containing two less carbons. The two carbons removed by β oxidation are split off as acetic acid.

As soon as the shorter fatty acid chain is formed, its β -carbon atom is attacked, and the process may continue step-wise until the final products are CO_2 and H_2O . According to the Knoop hypothesis, a long-chain fatty acid would be oxidized as follows:

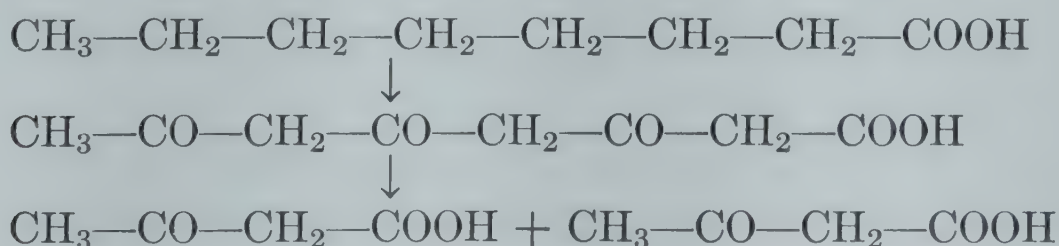


When butyric acid is oxidized, acetoacetic acid and β -hydroxybutyric acid (acetone bodies) are formed.

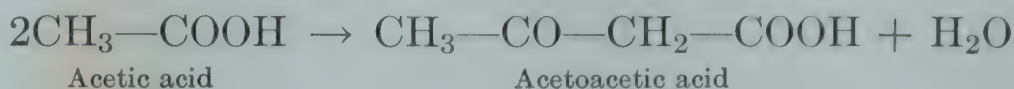


The β oxidation theory is not completely satisfactory for the reason that it fails to explain the formation of a number of degradation products that are known to be formed in fat metabolism. Nevertheless, it is probable that β oxidation is at least the first step in the oxidation of fatty acids.

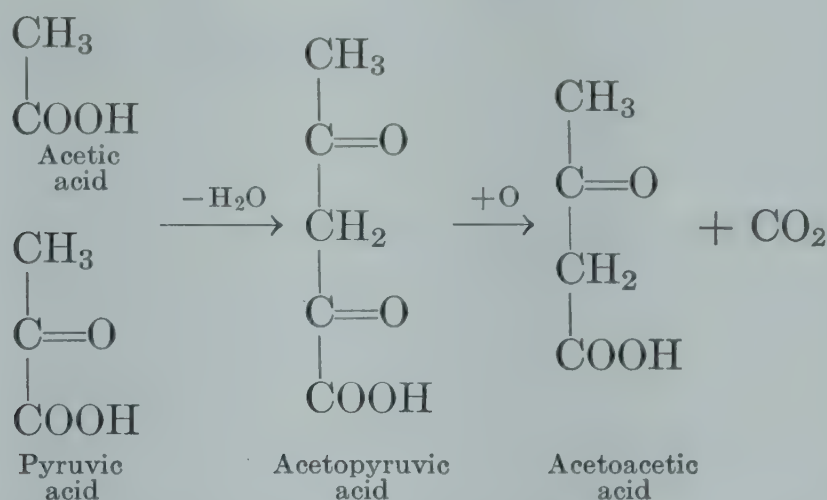
Multiple alternate oxidation. The β oxidation theory of Knoop, which we have just described, is characterized by the step-wise degradation of fatty acid chains, two carbons at a time. Jowatt and Quastel proposed the multiple alternate oxidation theory. These workers contend that more acetoacetic acid may be formed in fat metabolism than can be accounted for by the unmodified Knoop theory. As a result these workers visualize the formation of carbonyl groups on alternate carbon atoms, as follows:



β Oxidation condensation theory. This theory postulates that every fatty acid chain is oxidized at alternate carbon atoms and that the molecule then splits at each carbonyl group, thereby forming many molecules of acetic acid, except where a three-carbon chain is left. The three-carbon chain oxidizes to propionic acid, which is glycogenic rather than ketogenic. The theory goes on to postulate that pairs of acetic acid molecules finally condense to form acetoacetic acid as follows:



Krebs-Johnson theory. This theory differs slightly from the theory previously described, since it suggests that acetic acid and pyruvic acid can condense to form acetopyruvic acid, which, in turn, can be oxidized to acetoacetic acid.



Omega theory of oxidation. Although this theory cannot be overlooked, it is considered an emergency mechanism which is not the general pathway of fatty acid oxidation. Briefly described, this theory calls for the oxidation of the terminal methyl group, thereby forming a dicarboxylic acid. Consequently β oxidation could then set in at both ends of the dicarboxylic acid molecule.

Ketogenesis. When acetone bodies (acetoacetic acid and β -hydroxybutyric acid) are formed during metabolism, the process is known as *ketogenesis*. As a result compounds capable of forming acetone bodies are said to be *ketogenic compounds*. When an excess of these keto acids is formed or accumulates in the blood and tissues, the condition is known as *ketosis*. The

term *ketonemia* refers to excessive amounts of ketone bodies in the blood, and the presence of these compounds in urine is known as *ketonuria*. Under normal conditions, the body is able to utilize acetoacetic acid and β -hydroxybutyric acid. It is only when these are produced in excessive amounts that trouble occurs.

Since carbohydrates are oxidized preferentially, they are said to have a *fat-sparing* effect. Diets that are carbohydrate-rich and fat-poor are said to be *antiketogenic*. Diets that are rich in fats tend to overload the oxidative capacity of the tissues to the point where acetone bodies accumulate faster than the body can utilize them. In severe diabetes these acetone bodies accumulate in blood, tissues, and urine. This ketosis is sometimes called *acidosis*. In advanced cases of diabetes, acetone ($\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_3$) is formed and can be detected by the odor of the expired breath of the patient.

The threshold at which the keto acids spill over into the urine varies with individuals. As a rule, women and children are more susceptible to ketosis than men. The Eskimo normally subsists on fat-rich diets containing little, if any, carbohydrates. Nevertheless, ketosis is rare among these people. Evidently these races have become adapted to fat-rich diets by developing high ketosis thresholds.

Essential fatty acids. In 1930 Burr described experiments with rats which led to the conclusion that certain unsaturated fatty acids are indispensable for health and growth. These conclusions have been substantiated by subsequent researches. It is apparent that these unsaturated fatty acids must be furnished by the diet, since the body is unable to synthesize them. These acids are *linoleic*, *linolenic*, and *arachidonic*. Of these, linoleic acid seems to be the most effective, although any one of the three acids can meet the body requirements. When the diet is deficient in these unsaturated fatty acids, rats fail to grow, develop a characteristic dermatitis, and lose the power of reproduction. If the deficiency is permitted to progress, kidney lesions develop and death ensues.

Cholesterol metabolism. Although food (eggs, milk, and animal fats) furnishes some cholesterol, the amount ingested is not sufficient to account for the amount of cholesterol normally present in blood and body tissues. Therefore it is quite evident

that cholesterol must be synthesized in the body tissues. The liver is thought to be the seat of cholesterol synthesis. The rate of cholesterol synthesis is controlled by the cholesterol intake. Cholesterol production is at a maximum when cholesterol-free diets are fed, but cholesterol production falls to a minimum when cholesterol-rich diets are fed. The liver seems to be the principal mechanism whereby the rate of cholesterol production can be controlled. Excess amounts of liver cholesterol are excreted in the bile.

The role of fats as sources of heat and energy was discussed in Chapter 19.

22 · *Protein Metabolism*

In our study of digestion we learned that the final products of protein digestion are amino acids and certain chemical substances which are normal constituents of the conjugated proteins. These final products of protein hydrolysis are absorbed by the intestinal mucosa and are carried to the liver via the portal vein. Those amino acids which are not retained in the liver are transported by the systemic blood to the body tissues where they are utilized for the synthesis of proteins and other nitrogenous compounds.

It should be borne in mind that the proteins differ from carbohydrates and fats in that they function primarily as building material for the construction of body tissues. Immediately after a meal, the amino acid concentration rises in the portal blood, liver, and systemic blood. The concentration then falls gradually to the normal level, as the amino acids are taken up by the body tissues.

The amino acids are in a dynamic state of equilibrium with the body proteins at all times, regardless of the amount of protein in the diet. Tissue proteins are undergoing continuous synthesis and degradation. As a result proteins and amino acids are in a continuous state of flux.

Nitrogenous equilibrium. Proteins differ from carbohydrates and fats in that they cannot form large storage reserves or depots. In other words, protein storage capacity is limited. A part of the nitrogen of food proteins is not absorbed but is excreted in the feces. Absorbed nitrogen is metabolized and finally excreted in the urine as urea, creatinine, uric acid, and other waste products.

If the amount of nitrogen excreted in the urine and feces is equal to the amount of nitrogen eaten during a given period,

the animal is said to be in a state of *nitrogenous equilibrium*. Under these conditions the animal is neither gaining nor losing nitrogen. If the total nitrogen excreted exceeds the amount ingested, the animal body is losing nitrogen and is said to be in a state of *negative nitrogen balance*. This situation always occurs during starvation and in certain wasting diseases.

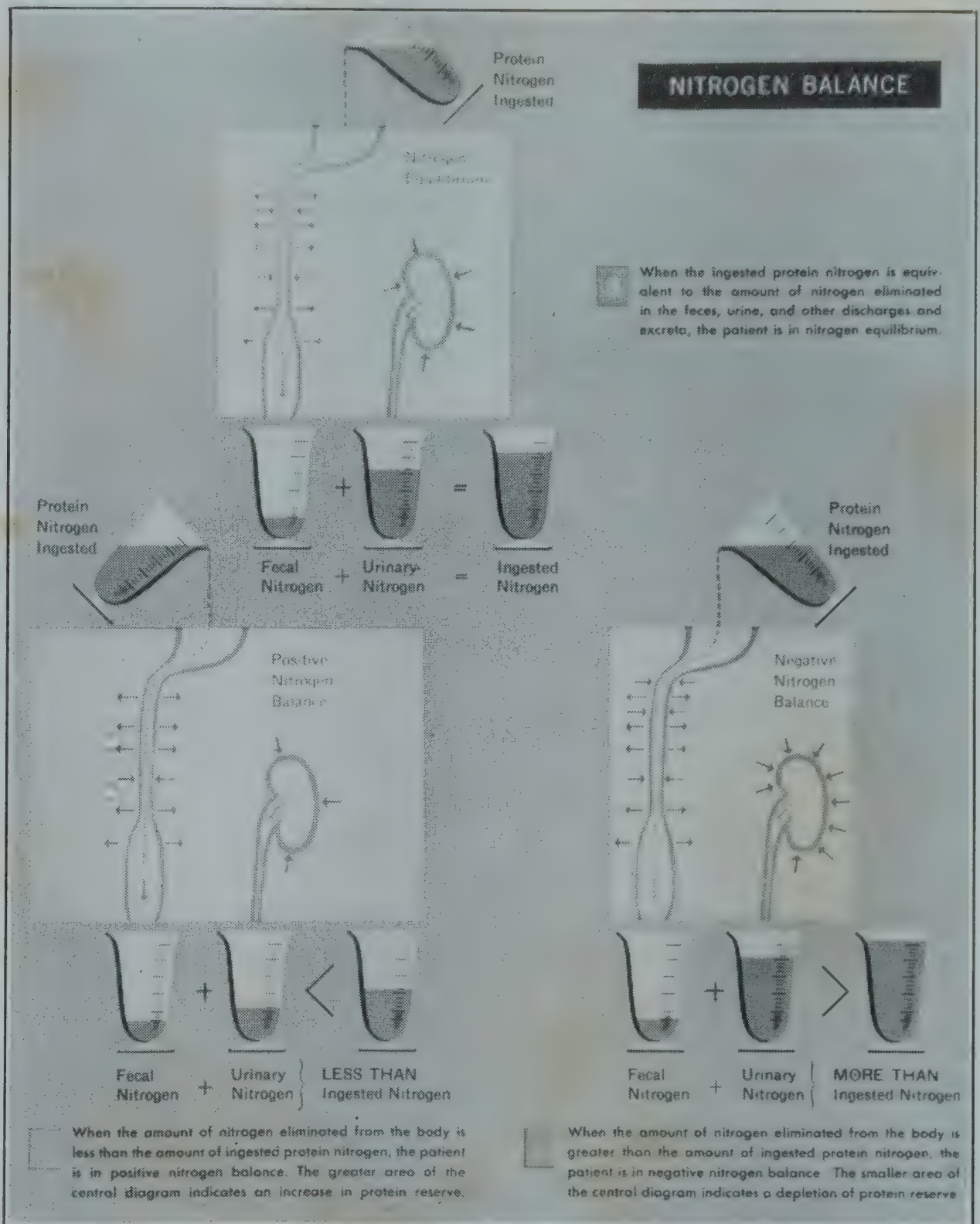


FIG. 22. Schematic diagram showing what is meant by (a) nitrogen equilibrium, (b) positive nitrogen balance, and (c) negative nitrogen balance. (Courtesy of Sharp and Dohme.)

When an animal is in nitrogenous equilibrium, the body weight tends to remain stationary, whereas negative nitrogen balance is accompanied by loss of body weight. When young animals are growing rapidly, the amount of ingested nitrogen is always greater than the amount of nitrogen excreted in the urine and feces. This is because the ingested nitrogen is being retained in the body for the construction of new tissues. This type of animal is said to be in a state of *positive nitrogen balance*.

By use of the nitrogen balance method it has been possible to measure the comparative efficiencies or biological values of individual purified proteins and of various protein-rich foods. In general, the biological value of a protein, determined by this method, may be expressed as the fraction or per cent of the ingested protein nitrogen actually used by the body. If all other dietary requirements are met, the minimum protein requirement, per day, is the least amount of a given protein which will permit an animal to remain in nitrogenous equilibrium. If, for example, twice as much of protein A as of protein B is required to keep the animal in nitrogenous equilibrium, it can be said that (weight for weight) the biological value of protein A is one-half or 50 per cent as valuable as protein B. Protein quality is even more important than protein quantity. Protein quality depends on the kind and amount of amino acids present. If all the essential amino acids are present in adequate amounts, the protein will possess high biological value. If certain essential amino acids are absent or present in low concentration, the protein will possess low biological value. Mitchell of the University of Illinois and Allison of Rutgers University have used the nitrogen balance method to determine the biological value of proteins, using whole egg protein as an arbitrary reference standard.

Nutrition workers are not in complete agreement regarding the best method of determining the biological value of proteins. Some workers believe that the maintenance method should be replaced by growth methods, since growth requirements are different from body-weight maintenance requirements. Nutrition and growth will be discussed in a subsequent chapter.

Fate of absorbed protein. Amino acids are not changed during absorption and are transported in the blood as free amino acids.

The fate of these acids is varied. Some are transformed into tissue proteins, others are broken down to furnish energy, CO_2 , H_2O and NH_3 , and others may serve as precursors, or as actual constituents, of hormones and other biological substances.

A few amino acids, which escape absorption, may be attacked by microorganisms in the lower digestive tract and changed to putrefactive products such as organic acids and amines. Some of these putrefactive products are toxic if absorbed in quantity.

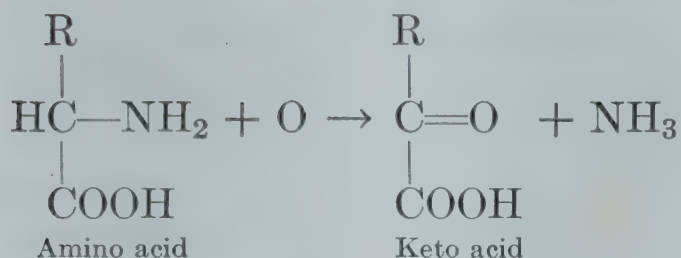
Protein storage and conservation. It has been stated that the animal body cannot store protein reserves in the sense that glycogen and fat are stored. Nevertheless, the body does possess the power of limited protein storage. When the protein intake is increased, there is not an immediate excretion of additional urinary nitrogen, indicating that the increased dietary nitrogen has "piled up" temporarily in the body.

Experiments show that liver, kidneys, and intestinal tissues increase in weight and in protein content after increased protein intake. Conversely, there is a comparable reduction in weight and in protein content of the above-mentioned organs when the protein intake of the animal is reduced.

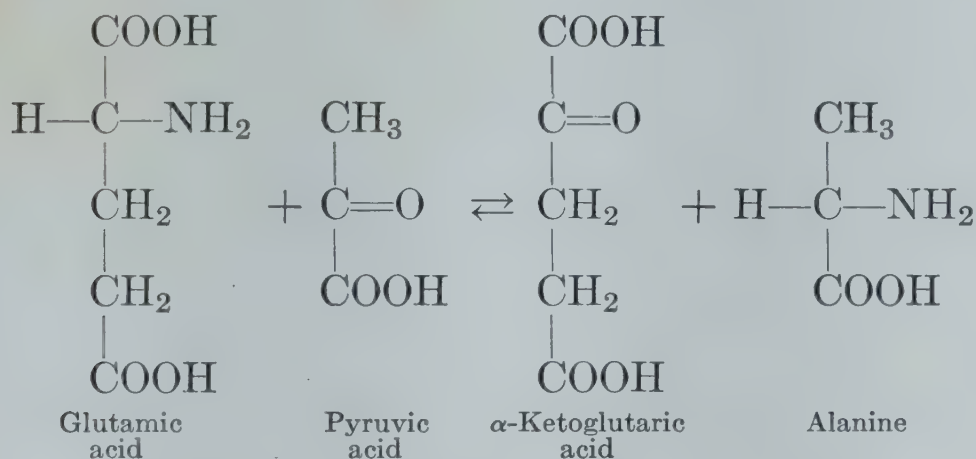
During high protein intakes the plasma proteins increase in volume. Conversely, plasma protein volume decreases during fasting. This leads to the conclusion that plasma proteins contribute to protein storage. In marked starvation the muscles waste away, thus contributing protein for the maintenance and prolongation of life. It is interesting to note that heart muscle wastes more slowly than other muscle tissues, which is nature's way of averting death as long as possible.

When animals fast, protein is conserved by calling first on the glycogen reserves. During the short period in which glycogen reserves are being depleted, nitrogen excretion is low. Consequently, stored carbohydrate is said to have "protein-sparing" properties. Moderate amounts of fats have a similar effect when the animal receives suboptimal protein intakes. However, diets rich in fat tend to aggravate the negative nitrogen balance.

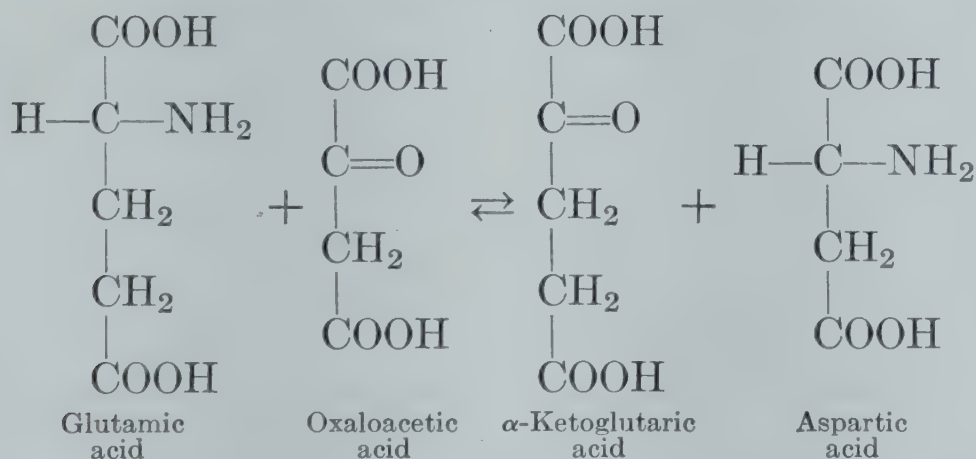
Deamination. When amino acids are metabolized, the initial step is the removal of the α -amino group. This is an oxidative reaction in which ammonia is formed, leaving a keto acid.



Transamination. When an amino acid is fed which contains the isotope N^{15} , the isotope is found in the amino acids of the proteins of the blood plasma and of the body tissues. This transfer of amino groups from one amino acid to another is called *transamination*. Glutamic and aspartic acids occur in muscle tissue in relatively large amounts and play an important role in transamination reactions. For example, glutamic acid can react with a common metabolite, pyruvic acid, to form the amino acid alanine.



Similarly, glutamic acid can react with another common metabolite, oxaloacetic acid, to form aspartic acid.



Thus it can be seen (1) that amino groups can be transferred from one compound to another and (2) that intermediate products of carbohydrate metabolism (pyruvic acid and α -ketoglutaric acid) also can play a role in protein metabolism.

This suggests a mechanism by which amino acids can be synthesized from carbohydrates. Many amino acids are capable of undergoing transamination. However, the amino acid, lysine, is an exception. By means of isotopes, it has been possible to show that the carbon chains of amino acids are also exchangeable. All these changes are controlled by enzyme systems, many of which have not been identified.

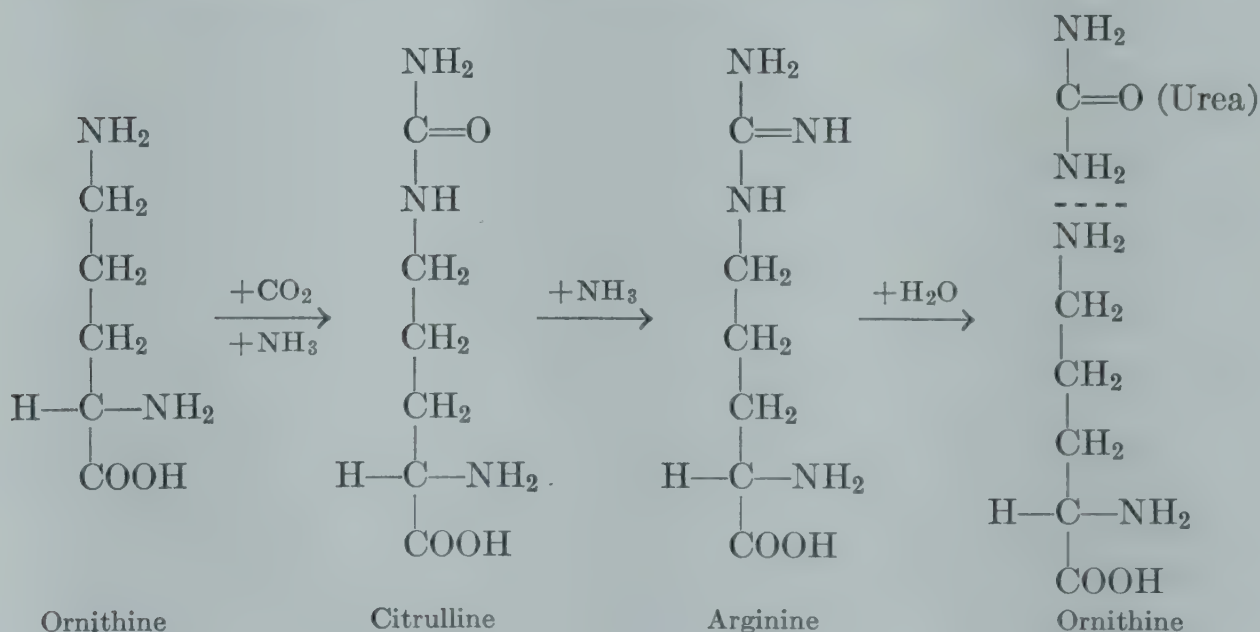
Urea formation. Urea is formed in the liver and is carried by the blood to the kidneys, where it is transferred to the urine and eliminated from the body as a nitrogenous waste product. Originally it was thought that urea formation was a simple process involving the formation of ammonium carbonate $[(\text{NH}_4)_2\text{CO}_3]$ from NH_3 and CO_2 followed by the formation of

$$\begin{array}{c} \text{O} \\ || \\ \text{NH}_4-\text{O}-\text{C}-\text{NH}_2 \end{array}$$

ammonium carbamate ($\text{NH}_4-\text{O}-\text{C}-\text{NH}_2$). It was thought that urea ($\text{NH}_2\cdot\text{CO}\cdot\text{NH}_2$) was formed from the carbamate by the loss of a molecule of water.

Evidence now indicates that urea synthesis is a much more complicated process. According to the Krebs-Henseleit theory, which has received support from recent studies with isotopes, ornithine, a diamino acid, unites with NH_3 and CO_2 to form citrulline, which combines with more NH_3 to form arginine. The latter compound is hydrolyzed by arginase, yielding urea and ornithine. Thus ornithine is regenerated to be used again and again for the synthesis of more urea.

These reactions can be summarized as follows:



The urea content of the urine of mammals depends on the protein intake. Excess nitrogen not needed for tissue building is excreted as urea. From 80 to 90 per cent of the total nitrogen of urine is in the form of urea.

It is interesting to note that only those animals (mammals, turtles, and certain fish) whose livers contain the enzyme, arginase, excrete urea as the principal end product of protein metabolism. Birds, lizards, and snakes possess no arginase and, as a result, do not excrete urea. Instead, their principal end product of protein metabolism is uric acid which is formed in the kidneys. Although the Krebs-Henseleit cycle is undoubtedly the principal method of urea formation, there may be, and probably are, other ways in which urea may be formed.

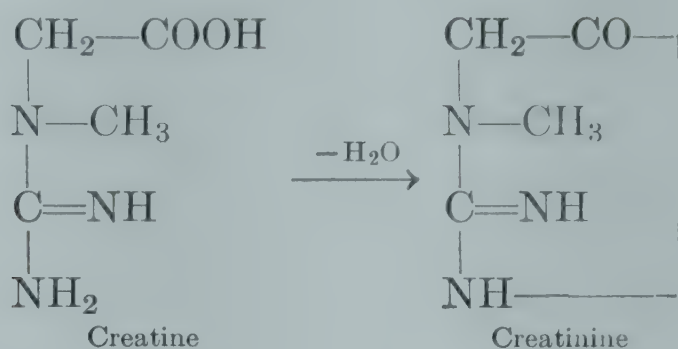
Formation of ammonia. In normal blood, the concentration of NH_3 is very low (0.1 to 0.2 milligram of ammonia nitrogen per 100 milliliters of blood). If the liver is removed, the NH_3 content of the blood rises to toxic levels, causing death. About 4 per cent of the total urinary nitrogen is in the form of ammonium salts. Urinary NH_3 arises as a result of deamination of amino acids. Work with isotopes has shown that urinary NH_3 does not occur as a urea degradation product, as was formerly postulated.

Fate of deaminized residues. A detailed discussion of the fate of non-nitrogenous residues of deaminized amino acids is not feasible in a book of this type. It is possible, however, to make a few generalizations. About eighteen of the more common amino acids are *glucogenic*; i.e., they are capable of forming glucose. About five amino acids are known to be *ketogenic*; i.e., they form keto acids.

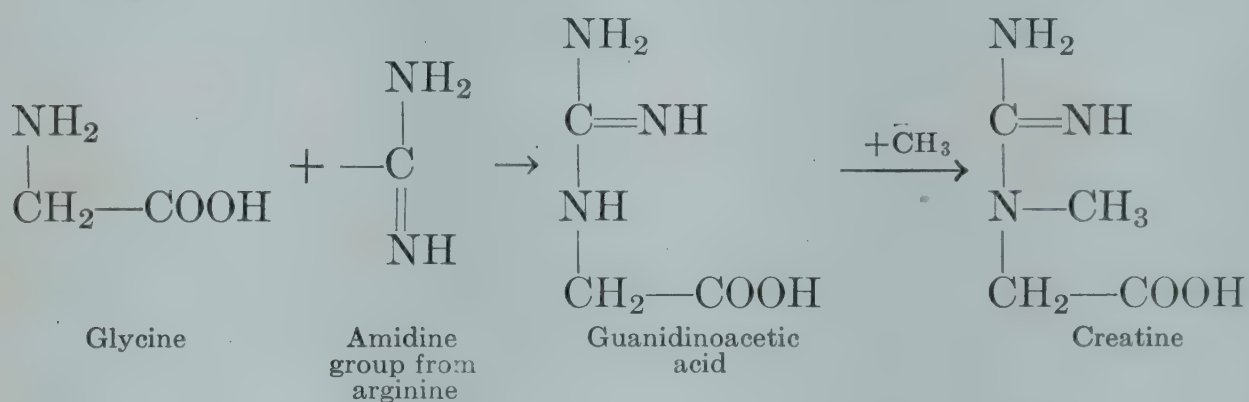
All the non-essential amino acids and a few of the essential amino acids are glucogenic. On the other hand, all the ketogenic amino acids are indispensable; i.e., they cannot be synthesized in the animal body and must be eaten, preformed, in the food. The essential amino acids will be discussed when we consider nutrition and growth.

Creatine and creatinine. These nitrogenous compounds are products of protein metabolism. Creatine is found in largest concentration in muscle tissues. Creatinine is a normal urinary waste product.

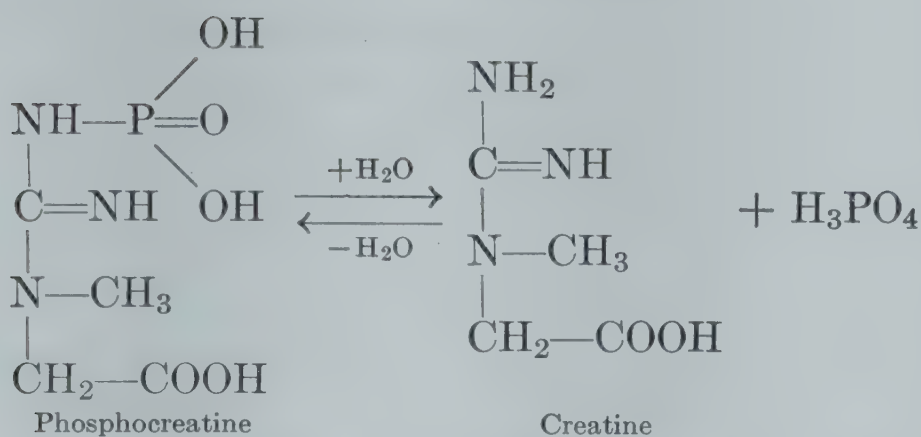
Creatine is methylguanidinoacetic acid. Creatinine is formed from creatine by loss of a molecule of H_2O , forming an inner anhydride of creatine.



The daily excretion of creatinine is remarkably constant for each individual and is not influenced by the protein intake or by muscular exercise. The level of creatinine excretion is related to the amount of musculature possessed by the individual. By use of isotopes, it has been established that urinary creatinine is derived from tissue creatine and that tissue creatine is synthesized in the body. These studies show, also, that the nitrogen of creatine is contributed by the amino acids, arginine and glycine. Arginine furnishes the amidine group ($\text{NH}=\overset{\text{I}}{\text{C}}\text{—NH}_2$) for the formation of the guanidine portion of creatine, and the remaining nitrogen is supplied by glycine. The methyl group in the guanidine nucleus is furnished by the labile methyl group of methionine. These changes can be shown as follows:



Creatine is present in muscle largely as phosphocreatine, an energy-rich compound which hydrolyzes to creatine and H_3PO_4 , accompanied by the release of energy.



Transmethylation. Choline and methionine contain labile methyl groups which can be transferred to other compounds. This transfer of methyl groups is called *transmethylation*. The synthesis of creatine from guanidinoacetic acid, cited in a previous paragraph, is an example of the methylating properties of methionine ($\text{CH}_3-\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{COOH}$). Choline (trimethylhydroxyethylammonium hydroxide) also contains labile methyl groups. These transmethylation reactions are important in the metabolic synthesis of creatine and choline, as well as in the metabolism of sulfur-containing amino acids. The vitamin, niacin (nicotinic acid), is methylated during metabolism and is excreted in the urine as N-methylnicotinamide.

CONJUGATED PROTEINS

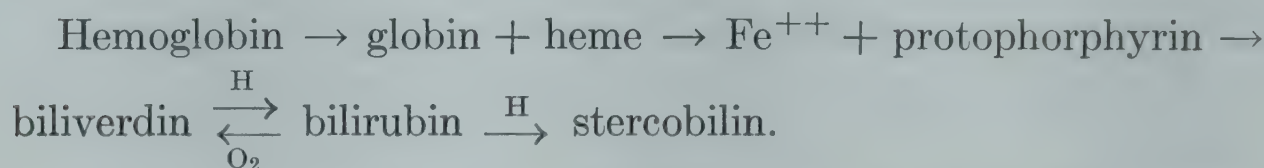
To this point, our discussion of protein metabolism has dealt with the simple proteins that yield only amino acids on hydrolysis. The conjugated proteins differ from the simple proteins in that they yield other chemical substances in addition to amino acids.

Some of these other hydrolytic products are carbohydrates, fats, pigments, and nucleic acids. Examples of conjugated proteins are (1) *glycoproteins*, which contain a sugar or sugar derivative; (2) *lipoproteins*, which contain a lipid fraction; (3) *chromoproteins*, which contain such substances as hemoglobin, hemocyanin, ferritin, and cytochrome-C, and (4) *nucleoproteins*, which contain nucleic acids. The most important conjugated proteins are the chromoproteins and the nucleoproteins. The protein fractions of the conjugated proteins are digested, absorbed, and metabolized like the simple proteins,

but the prosthetic groups undergo changes which are characteristic of the chemical structures involved.

Chromoproteins. All chromoproteins contain *porphin*, which is a complicated structure containing four pyrrole rings (see Chapter 17). Hemoglobin is a chromoprotein in which *heme*, the prosthetic group, contains ferrous iron as a part of the porphin nucleus. The protein, *globin*, is attached to the iron atom. *Reduced hemoglobin* unites with oxygen to form *oxy-hemoglobin*, in which form it acts as an oxygen carrier in the blood.

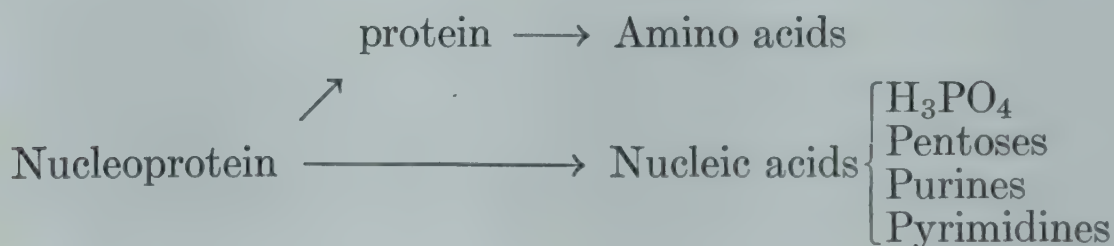
When hemoglobin is decomposed in the liver, the spleen, and bone marrow, iron is removed and stored. Biliverdin, the green bile pigment, is formed in the liver from protoporphyrin, which remains after the iron is removed. Bilirubin, the yellowish red bile pigment, formed by the reduction of biliverdin, is changed to a brown pigment, stercobilin, by the action of intestinal bacteria. These steps can be summarized as follows:



Nucleoproteins. Like other conjugated proteins, the protein portion of the nucleoproteins passes through the same metabolic cycle as that described for the simple proteins. The prosthetic group, *nucleic acid*, undergoes an entirely different series of chemical changes.

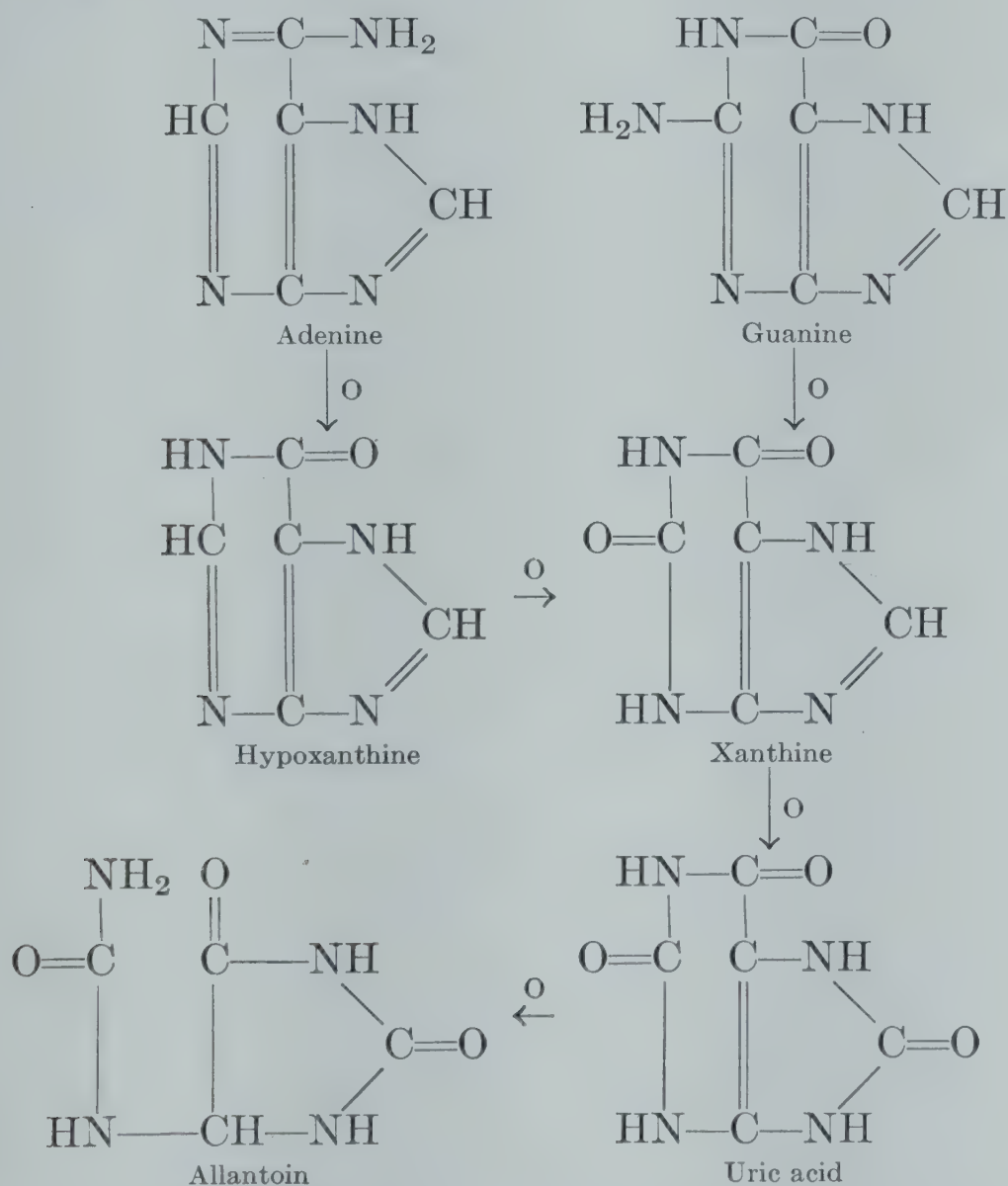
Nucleic acids are split to *purine nucleotides* and *pyrimidine nucleotides* by the action of nucleic acidase. The nucleotides are split to nucleosides and H_3PO_4 by nucleotidase. Nucleosides, in turn, are hydrolyzed by nucleosidases to form a pentose sugar and purines or pyrimidines. The pentose sugar fraction usually consists of D-ribose or D-2-desoxyribose.

The following diagram summarizes the hydrolytic steps just described:

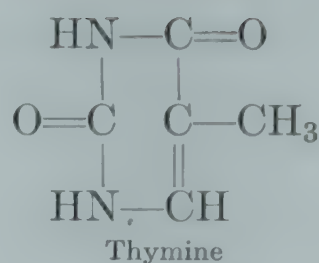
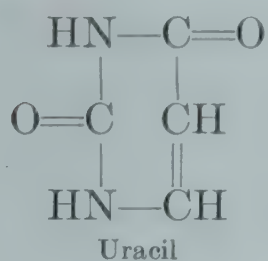
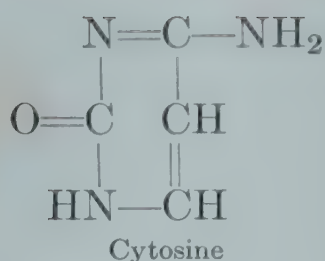


The carbohydrate and phosphate fractions follow the usual metabolic paths; the purines, *adenine* and *guanine*, are oxidized to *uric acid* or *allantoin*, depending on the species of animal. Uric acid is the principal product of purine metabolism in humans, Dalmatian dogs, and anthropoid apes. In other species the uric acid is changed to allantoin, in which form it is excreted.

Purines. Adenine is 6-amino purine, and guanine is 2-amino-6-oxypurine. During metabolism the amino groups are replaced by oxygen atoms, forming *oxypurines*. Adenine forms *hypoxanthine* (6-oxypurine), and guanine forms *xanthine* (2,6-dioxy-purine). Hypoxanthine oxidizes to form xanthine, and xanthine oxidizes to form uric acid (2,6,8-trioxypurine). The following scheme summarizes the steps in purine metabolism:



Pyrimidines. Some nucleic acids yield pyrimidines on hydrolysis. Examples are *cytosine*, *uracil*, and *thymine*.



Very little is known regarding the metabolism of the pyrimidines. It is probable that the pyrimidines are synthesized from the products of protein degradation and that the purines are derived from the pyrimidines. In spite of the fact that the purines and pyrimidines contain urealike structures (N—C—N), all evidence points to the fact that urea is not a precursor of these compounds.

23 · Protein Nutrition

Energy and vitamin requirements of man and domestic livestock have been discussed in preceding chapters. We will now consider the relationships of protein quality and quantity to animal nutrition.

Protein quality. In our discussion of foods and feeding stuffs, it was stated that proteins contain, on the average, about 16 per cent nitrogen. This has been common knowledge for more than 80 years. As a result, most early workers believed that all food proteins were of equal nutritional value. However, a few pioneer investigators questioned this assumption.

Voit, for example, was able to show as early as 1872 that dogs could be maintained in nitrogenous equilibrium on a diet of lean meat but that the same dogs went into a negative nitrogen balance when meat was replaced by gelatin. He concluded that gelatin was nutritionally inadequate when fed as the sole source of dietary nitrogen.

In 1913, Abderhalden described an experiment in which a dog had been maintained in good health for 100 days on a diet containing a protein-free meat hydrolyzate as the sole source of dietary nitrogen. During the experiment, the dog actually gained about 20 pounds in body weight. This led Abderhalden to conclude that protein, of itself, is not necessary for tissue building and that body tissues can be synthesized from protein degradation products (amino acids). This was contrary to prevailing concepts, since most authorities of that period believed that food proteins were absorbed and utilized as proteins. Chemists cited the existence of the "circulating proteins" of blood in support of this theory.

Growth of the amino acid conception. Subsequent work by Willcock and Hopkins, Kaufman, Folin and Denis, Van Slyke and Myers, Abel, and others finally led to the conclusion that

digested proteins are absorbed as amino acids and that these acids serve as building stones in the repair of old tissues and in the building of new tissues. Evidence was also accumulating to indicate that the differences in nutritive value of good and poor proteins might be explained on the basis of their amino acid content. Osborne and Mendel were able to show, by means of feeding experiments with rats, that the growth-promoting properties of proteins were dependent on the amino acid content

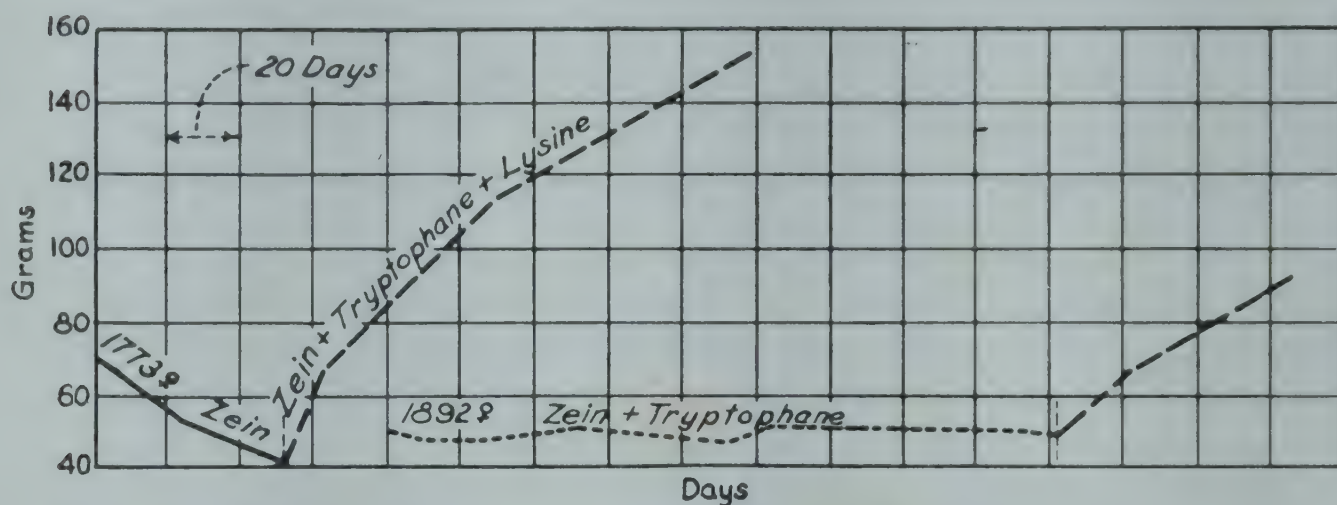


FIG. 23. Showing the biological inadequacy of the protein zein (from corn). This protein will not support growth until it is supplemented with the amino acids tryptophan and lysine. (Data of Osborne and Mendel.)

of the proteins. Zein, from corn, did not promote growth because the amino acids lysine and tryptophan were lacking. When these amino acids were added to the inadequate zein-containing diet, growth ensued. Gliadin, a protein from wheat, required additions of lysine before growth was satisfactory. On the other hand, casein from milk produced excellent growth when fed at suitable levels.

As a result of these and similar studies, the conviction grew that casein, lactalbumin, and meat proteins are biologically superior to certain vegetable proteins because they contain certain amino acids which are essential for tissue synthesis and which must be furnished preformed in the food because the body cannot synthesize them. As a result, it was postulated that animal proteins tend to be biologically superior to vegetable proteins. There are exceptions to this generalization, but sub-

sequent work has shown that the most complete proteins are of animal origin.

Amino acid requirements of the rat. Many attempts had been made to feed artificial mixtures of crystalline amino acids as a substitute for dietary protein in rat diets. These experiments were not successful, since growth could not be obtained and animals died if the experiments were prolonged.

Until 1931, only three amino acids (lysine, tryptophan, and histidine) were thought to be essential. At that time, Rose of Illinois described his attempts to rear rats on diets in which the sole source of nitrogen was derived from mixtures of all known amino acids. Like workers who had preceded him, he also failed to obtain satisfactory growth. He was convinced that his amino acid mixture was lacking in an amino acid (or acids) that had not yet been isolated or identified. He proved the correctness of this assumption by isolating and identifying a hitherto unknown amino acid (α -amino- β -hydroxybutyric acid) which he named *threonine*. When this amino acid was added to his amino acid mixture, rats no longer lost weight or died, and fair growth for short periods was obtained. This was the first successful attempt to grow experimental animals on diets in which the dietary nitrogen was derived solely from amino acid mixtures.

Rose then conducted experiments in which he omitted one amino acid at a time from the dietary mixture. If the omission of an amino acid did not affect the growth rate, this amino acid was not considered essential. If, however, growth was depressed by the removal of an amino acid and if growth was resumed by the replacement of the acid, the acid was considered *essential* or *indispensable* for growth.

Rose and co-workers finally established the fact that ten of the twenty-two commonly occurring amino acids are essential for life and growth. The twelve remaining amino acids can be synthesized in the animal body. Rose has defined *an essential amino acid as one that cannot be synthesized by the animal organism out of materials ordinarily available at a speed commensurate with the demands of normal growth*.

The following table lists the essential and non-essential amino acids as determined by the Rose method:

ESSENTIAL AMINO ACIDS	NON-ESSENTIAL AMINO ACIDS
Arginine	Alanine
Histidine	Aspartic acid
Isoleucine	Citrulline
Leucine	Cystine
Lysine	Glutamic acid
Methionine	Glycine
Phenylalanine	Hydroxyproline
Threonine	Proline
Tryptophan	Serine
Valine	Tyrosine

Rose and co-workers found that arginine can be synthesized by the rat but at a rate insufficient to supply the total arginine requirement.

Other species. It appears that the chick differs from the rat, since the former cannot synthesize arginine and glycine, and the rate of synthesis of proline and glutamic acid is too slow for normal requirements. The amino acid requirements of cats, dogs, and swine appear to be quite similar to those of the rat. Ruminants, on the other hand, are able to synthesize amino acids and proteins by means of microorganisms which grow in profusion in the rumen or paunch. Even non-protein sources of nitrogen, such as urea and ammonia, have protein replacement value in ruminant nutrition, since rumen microorganisms are able to synthesize bacterial protoplasm from these non-protein materials. If sufficient carbohydrates are present in a low-protein ration to furnish energy for bacterial growth, ruminants can make fairly efficient use of the nitrogen of urea and ammonium salts.

If, however, the protein content of the ration is adequate (16 to 18 per cent), no benefit is derived by adding urea or ammonium salts. Use of urea and similar non-protein sources of nitrogen may be said to be still in the experimental stage, and it is doubtful if they will play an important role in ordinary feeding practice.

Amino acid requirements of man. Recently, Rose and co-workers have shown that the amino acid requirements of man are similar to but not identical with those of the rat. Using more than forty male graduate students as experimental subjects,

Rose and his associates have conducted human feeding experiments in which crystalline amino acids were the sole source of dietary nitrogen. The diet, which was far from appetizing, consisted of amino acids, starch, cane sugar, butterfat, inorganic salts, and vitamins. The amino acids were administered in water, flavored with sugar and lemon juice. Starch, salts, and most of the butterfat were mixed with water and baked into wafers. The remaining butterfat was used as a "spread" for the wafers. Vitamins were ingested in the form of pills.

Nitrogen balance studies, rather than growth response, were used to determine the biological efficiency of the amino acid mixtures. When the amino acid mixture contained all the essential amino acids, experimental subjects had no difficulty in maintaining nitrogenous equilibrium. If, however, an essential amino acid was removed from the mixture, the subjects went into negative nitrogen balance.

By this method, Rose was able to show that, with the exception of arginine and histidine, the amino acid requirements of man were qualitatively identical with those of the rat. As a result it can be stated that present data indicate that *adult human beings require but eight essential amino acids, whereas ten amino acids are essential for the young growing rat.*

Daily human requirements. Rose has also attempted to determine the minimal amount of each essential amino acid required to maintain nitrogenous equilibrium in man. The following table lists (1) the minimum daily amounts required to maintain nitrogenous equilibrium and (2) the recommended daily allowances that will ensure an adequate supply of each amino acid.

ESSENTIAL AMINO ACIDS PER KILOGRAM OF BODY WEIGHT

Amino Acid	Minimum Daily Require- ment, gm	Recommended Daily Allow- ance, gm
L-Tryptophan	0.15–0.25	0.50
L-Phenylalanine	0.80–1.10	2.20
L- or D-Methionine	1.10	2.20
L-Lysine	0.40–0.80	1.60
L-Valine	0.80	1.60
L-Leucine	0.50–1.10	2.20
L-Threonine	0.50	1.00
L-Isoleucine	0.70	1.40

The so-called daily allowance is set sufficiently high to ensure adequate amino acid supplies, regardless of physiological variability of individuals.

Biological value of proteins. Although there are a number of methods for estimating the biological value of proteins, the simplest method is the nitrogen balance method. This method measures the efficiency of a given protein to furnish essential amino acids for the repair and synthesis of body tissues. Expressed in its simplest form, the biological value of a protein is the percentage of total absorbed nitrogen which is actually stored in the body. This is made clear by the following calculation:

$$\text{N intake} - \frac{(\text{Fecal N} + \text{Urinary N})}{\text{N intake} - \text{Fecal N}} \times 100 = \text{Biological value}$$

Different methods from different laboratories yield different absolute values, but all methods are in general agreement so far as high and low biological values and their intermediate gradations are concerned. It should be borne in mind that biological values for proteins also vary with the type of experimental animal used in making the measurements. The following data, by Sahyun, are estimates of the biological values of some food proteins for human adults expressed in arbitrary units. This author based his figures on "what is known and surmised" regarding the amino acid composition of these foods:

Whole egg	78	Rolled oats	60
Milk	74	Whole wheat	55
Meat	72	Corn meal	43
Soy flour	65	White flour	41

Amino acid content of proteins. Methods for the determination of amino acids still leave much to be desired. The procedures for hydrolyzing proteins must be chosen with great care in order that amino acids are not modified or destroyed during the hydrolysis. The following table shows the approximate percentage composition of some typical food proteins:

APPROXIMATE PERCENTAGE COMPOSITION OF TYPICAL PROTEINS

	Casein	Gelatin	Beef Muscle	Zein (Corn)	Gliadin (Wheat)
Arginine	4.3	9.2	7.2	1.6	3.0
Histidine	2.1	0.9	2.9	0.8	2.0
Lysine	7.6	5.3	8.2	1.3
Tyrosine	6.7	0.3	4.4	5.4	3.1
Tryptophan	1.2	...	1.4	0.1	1.0
Phenylalanine	5.0	2.4	5.0	7.0	...
Cystine	0.35	0.2	1.1	0.9	2.6
Methionine	3.4	1.1	3.4	2.4	3.0
Threonine	3.8	1.7	5.0	2.5	3.0
Serine	7.7	3.7	5.5
Leucine	9.7	3.6	7.7	25.0	...
Isoleucine	6.3	1.2	6.3	4.0	...
Valine	6.5	2.7	5.8	3.0	...
Glutamic acid	23.3	11.6	15.6	36.6	47.0
Aspartic Acid	6.1	9.6	6.1	3.4	1.4
Glycine	0.5	26.6	5.1	...	1.8
Alanine	5.5	10.4	5.0	10.0	5.8
Proline	8.0	17.2	...	12.0	13.0
Hydroxyproline	?	15.0

Examination of the above table shows that casein and beef muscle contain a much better distribution of the essential amino acids than is found in the less valuable proteins, gelatin, zein, and gliadin. Thus it can be seen that proteins can supplement each other in furnishing a better mixture of essential amino acids. This is one of the reasons why nutritionists advocate mixed diets, since such diets are more likely to contain a better distribution of all essential nutrients.

Protein supplementation. It is good feeding practice to supplement grain rations with *sources of animal protein* such as meat scraps, fishmeal, tankage, and milk products. The addition of these feeding materials not only improves the quality of the protein in the ration by furnishing essential amino acids; it also improves the nutritive value of the ration by adding other essential nutrients such as mineral salts and vitamins.

When animal protein feeds are not available or are too expensive, it is possible to supplement grain rations with protein feeds of vegetable origin, such as meals prepared from such materials

as soybean, cottonseed, linseed, peanut, sunflower, and the leafy fractions of alfalfa. Additions of any of these concentrates improve the protein quality of a grain ration. However, most successful feeders insist on animal protein supplements whenever supplies are available at prices they can afford to pay. It should be borne in mind that a serious deficiency of amino acids can cause failure of the entire ration, regardless of the nutritive superiority of the other ration ingredients. This is true, of course, for a deficiency of any highly essential ingredient, thus emphasizing the truth of the old saying that "no chain is stronger than its weakest link."

Protein hydrolyzates. Within the past few years medical and nutrition workers have been searching for suitable sources of amino acids that can be administered in predigested form for patients who, for one reason or another, are unable to digest food proteins. Considerable progress has been made in this field (diet therapy) through the use of protein hydrolyzates prepared from lactalbumin, casein, and blood fibrin. These have proved most useful in saving the lives of persons in advanced stages of starvation, where the gastrointestinal tract has ceased to function. Hydrolyzates can be administered orally or intravenously.

Nutrition (or hunger) edema often accompanies starvation. This condition is characterized by an accumulation of tissue fluids, accompanied by swelling of tissues. Edema usually occurs when people have been forced to subsist for long periods on low caloric intakes and subnormal protein and mineral intakes. This causes a fall in the concentration of plasma proteins and an abnormal mineral balance. Such cases have been treated successfully by injection or by careful initial feeding of protein hydrolyzates. During World War II malnourished prisoners and refugees were treated successfully, using careful initial feedings of a liquid made with powdered eggs, powdered milk, sugar, and water.

Hydrolyzates have been used in the treatment of surgical shock due to loss of blood and tissue proteins and in hastening the healing of wounds, fractures, and burns. The use of blood plasma is another method of replenishing body proteins.

Protein allowances. It should be remembered that there is a difference between *dietary requirements* and *dietary allowances*. It is practically impossible to specify the minimal requirement for any specific nutrient, for the reason that individuals within a species differ appreciably in efficiency of digestion, absorption, and utilization of the various nutrients. For this reason, most authorities are unwilling to express nutrient needs in terms of minimal requirements. It is understandable that the minimal requirements for one person might be subminimal for another person. As a result, most authorities prefer to allow for a "margin of safety," by specifying a nutrient intake that will ensure optimal nutrition. These optimal intakes are called *recommended allowances*.

Protein allowances for humans. The Food and Nutrition Board of the National Research Council recommends that the daily protein allowance for an adult male shall be 70 grams. It is recognized that man can subsist on much lower protein intakes when necessary. The daily allowance for women is 60 grams of protein, which should be increased to 85 grams during the latter half of pregnancy and to 100 grams per day during lactation.

Protein allowances for children range from 3.5 grams per kilogram (2.2 pounds) of body weight (for children under 1 year of age) to 40 grams per day (for children 3 years of age). From that point, the daily allowance is increased at a rate of about 10 grams per day for each 3-year age increment, up to 12 years of age, when the daily protein intake should reach 70 grams.

For girls from 12 to 20 years of age, the daily allowance ranges from 75 to 80 grams of protein; active boys of the same age should receive from 85 to 100 grams of protein per day.

Protein allowances for dairy cattle. Recommended allowances for domestic livestock have been made by the Committee on Animal Nutrition of the National Research Council. For dairy cattle the protein recommendations are expressed in terms of *digestible protein*, and growth allowances are based on body weight. For young growing stock, weighing 100 pounds, the daily allowance is 0.45 pound of digestible protein (D.P.) per day. As body weight increases, the daily D.P. allowances are as follows: 200 pounds, 0.70; 400 pounds, 0.80; 600 pounds,

0.85; 800 pounds, 0.90; 1000 pounds, 0.95; and 1200 pounds, 1.00. The need for digestible protein for maintenance of body weight is less than for growth. For animals maintained at 1000 pounds body weight the D.P. daily allowance is 0.60 pound, and, for 1200 pounds, 0.80 pound. During the last 6 to 12 weeks of pregnancy, dairy cows should receive 1.2 pounds of digestible protein per day.

For milk production, the digestible protein allowances are based on *body weight, pounds of milk produced, and the percentage of butterfat in the milk*. The following pounds of digestible protein must be added to the "weight allowances" for each pound of milk produced: milk with 3 per cent fat, 0.04 pound; 4 per cent fat, 0.045 pound; 5 per cent fat, 0.05 pound; and 6 per cent fat, 0.055 pound.

Protein allowances for beef cattle. Growing heifers and steers should receive 0.90 pound of digestible protein per day. Moderately active bulls require up to 1.4 pounds per day. Standards for fattening range from 1.1 to 1.8 pounds D.P. per day, depending on the age and body weight at time of fattening.

Protein allowances for swine. Allowances for swine are expressed in terms of *pounds of protein in the feed*. Young growing and fattening pigs should receive 0.60 pound of crude protein per day. By the time the body weight has reached 200 pounds, the crude protein intake should be 1.00 pound of protein per day. Intakes for lactating sows and breeding boars should range from 1.50 to 2.30 pounds of protein per day.

Protein allowances for poultry. Nutrients required for poultry feeding are expressed in terms of *percentage of the nutrient or ingredient in the ration* or in terms of *weight of nutrient per pound of feed*. Protein allowances are usually expressed in terms of percentage composition. For example, the protein allowance for starting chicks is a feed containing 20 per cent protein. The protein can be reduced to 16 per cent when the birds are 12 weeks of age, and laying and breeding hens can function efficiently on feeds containing 15 per cent protein. Young turkeys, on the other hand, require a feed containing 24 per cent protein. This can be reduced to 18 per cent at 12 weeks of age, and breeding stock will develop normally on feeds containing

15 per cent protein. (See Appendix for tables of nutrient allowances.)

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24 • *Míneral Metabolism*

In a previous chapter we learned that the inorganic portion of soil consists of chemical degradation products formed by the disintegration of rocks. Many of these form inorganic cations and anions which are utilized by growing plants and deposited in the plant tissues (leaves, seeds, fruit, and stalk). These tissues are sources of food for man and domesticated animals.

Many of these mineral elements are also essential for animals. The most important, from the standpoint of animal nutrition, are Ca, P, Na, K, Mg, Fe, Cl, S, I, Mn, Cu, Co, F, and Zn. Other elements are normal constituents of animal tissues, but information is lacking regarding their importance and function. Such elements are Si, Al, Br, As, and Ni.

Functions of mineral elements. The essential elements function in the animal body (1) as structural constituents of bones and teeth; (2) as constituents of organic compounds which are essential parts of the softer tissues such as muscle and blood cells, and (3) as soluble electrolytes in the body fluids which assist in controlling osmosis, acidity and alkalinity.

The elements Ca, Mg, Na, K, and Fe exist in food materials as salts of organic and inorganic acids. In plants, the organic salts may be malates, citrates, tartrates, or phytates, and the inorganic salts may be sulfates, chlorides, and phosphates. In milk, calcium is combined largely as calcium caseinate, and iron is present in meats largely as hematin. Sulfur usually enters the body in organic combination as protein or as sulfur-containing organic compounds which are found in such foods as cabbage, broccoli, and kale. Phosphorus may be ingested as phosphoproteins, nucleoproteins, phospholipids, hexose phosphates, inositol phosphates (phytic acids), and inorganic phosphates.

Calcium. In a previous chapter it was stated that calcium exists in the animal body in larger amounts than any other mineral element. Ninety-nine per cent of the body calcium is found in the skeletal tissues (bones and teeth), and the remainder exists partly in ionic form in the body fluids and tissues, in which form it plays an important role in maintaining normal activity of nerves and muscles, in the clotting of blood, and in influencing permeability of cells.

Soluble calcium salts are absorbed in the intestinal tract and carried by the blood to the tissues where calcium seems to be essential for normal cell function. It would appear that about one-half of the blood calcium is in ionized form, and the remaining calcium is loosely combined, probably with plasma protein.

A number of factors affect calcium absorption in the intestine. It may form insoluble carbonates, sulfates, phosphates, or oxalates which are not absorbed but which are excreted in the feces. Calcium can also form insoluble soaps with fatty acids. Thus, dietary calcium can be lost through the formation of unabsorbed compounds.

Consequently, the efficiency of calcium absorption depends on (1) the amount and form of calcium salt ingested; (2) the pH of the intestinal contents; (3) the presence of phosphates, sulfates, carbonates, and fats, and (4) the amount of vitamin D in the diet.

Bony tissues are continuously being torn down and rebuilt. As a result, the complex bone salts and the blood calcium are in a continuous state of flux. Under normal conditions the calcium content of blood is relatively constant in order to preserve this vital equilibrium. If, however, the calcium intake falls to low levels, the bones will decalcify and give up their calcium in order to maintain the calcium content of the blood. This weakens and softens the bones and produces skeletal abnormalities (see rickets and osteomalacia). The same phenomenon occurs in the growing teeth of young animals. Adult teeth, once formed, are not susceptible to this type of decalcification. This is why children and young growing animals have a high requirement for calcium and vitamin D.

The calcium level in the blood is regulated by *parathormone*, a hormone produced by the parathyroid glands. If the parathy-

roid glands are removed, the blood calcium level falls to a point where tetany occurs. These spasms can be relieved, but not cured, by the ingestion of soluble calcium salts. Metabolic calcium is excreted, eventually, in the urine as soluble calcium salts.

Phosphorus. From a quantitative standpoint, phosphorus is the second mineral element of importance so far as animal tissues are concerned. About 80 per cent of the total phosphorus of the body is found in bones and teeth. Phosphorus enters the body in many forms. These include organic esters, phosphoproteins, nucleoproteins, phospholipids, and inorganic phosphates. Because of the role played by phosphates in bone formation, vitamin D also plays a part in phosphorus utilization. Diets are often deficient in calcium, but this is less likely to be true of phosphorus. As a result, phosphorus deficiencies are less likely to occur than calcium deficiencies.

Although the dietary levels of calcium and phosphorus are important, the ratio of calcium to phosphorus is equally important. If calcium is low and phosphorus is high in the diet, rickets results. This is known as *low-calcium rickets*. If the situation is reversed, rickets also results. This is known as *low-phosphorus rickets*. In rapidly growing animals and children the Ca:P ratio should not be wider than 1:1 or 1:2. Experimental rickets-producing rations usually have Ca:P ratios of 8:1 or 6:1. Rachitogenic rations can be made less rachitogenic by feeding vitamin D, since this vitamin has the property of increasing calcium and phosphorus utilization.

In our discussion of the metabolism of fats, carbohydrates, and proteins, we learned that phosphates play important roles in metabolism by forming essential intermediary compounds and by serving as constituents of certain coenzymes. In addition to the roles already described, phosphates also play a part in regulating acid-base balance in blood and tissues.

Some dietary phosphorus can be lost by forming insoluble calcium phosphates in the digestive tract. This is particularly true of the digestive tract of herbivora where there is a tendency for alkaline plant residues to be present in relatively large amounts. Phosphates resulting from tissue metabolism are excreted as soluble phosphates in the urine.

Sodium. Sodium enters the body largely as sodium chloride and as organic and inorganic salts. This element contributes the greatest amount of base to blood and body tissues. Combined as phosphates, sodium plays important roles in maintaining osmotic and ionic equilibria. Sodium salts also serve to keep plasma proteins in solution. Inorganic salts, including sodium salts, are lost from the body through excessive perspiration. Collapse caused by excessive salt losses in perspiration can be prevented by the administration of salt tablets or by adding common salt to the drinking water. The average human being consumes about 10 grams of sodium chloride per day, which is in excess of the body requirements. Salts not needed for physiological purposes are excreted in the urine.

Potassium. Potassium salts are present in plant and animal tissues as salts of inorganic and organic acids. In the cells of the animal body, potassium is combined as phosphates, chloride, and bicarbonate. Red blood cells contain approximately thirty times as much potassium as blood serum, and similar ratios exist between the potassium content of tissue cells and the surrounding tissue fluids. It appears that potassium or its salts are capable of forming loose chemical combinations with the organic constituents of cells. When potassium is omitted from purified experimental diets, growth failure occurs, followed by paralysis. The daily requirement of animals and man for potassium is not known.

Chlorine. Since foods are reasonably rich in chlorides and since common salt is easily available, there is little chance for the occurrence of chlorine deficiencies in animals or in man. Exceptions may occur in cases of profuse sweating or in diarrhea. About two-thirds of the anions of blood consists of chlorine ions. Cerebrospinal fluid contains more chlorine than any other body fluid or tissue. This element is also present in hydrochloric acid in gastric juice. About 90 per cent of ingested chlorine is excreted in the urine, perspiration accounts for 4 per cent, and feces, 1 per cent.

Magnesium. Approximately 75 per cent of the magnesium in the body exists in combination with calcium and phosphorus in the complex bone salts. The remaining 25 per cent is found in the soft tissues and body fluids. Magnesium can replace

some of the calcium in bone, but the process is not physiologically efficient or desirable. Magnesium deficiency has been produced experimentally in rats by McCollum and co-workers, but there is little evidence that magnesium deficiency occurs in domestic animals or in human beings. Magnesium salts are excreted in the urine.

Sulfur. This element is a normal constituent of all body cells, where it exists as sulfur-containing amino acids in the tissue proteins. These amino acids are cystine, cysteine, methionine, and ergothioneine.

Chondroitin sulfuric acid, in cartilage and bone, contains sulfur in combination with carbohydrates. Smaller amounts of sulfur occur, combined in such important compounds as thiamine, glutathione, biotin, and insulin. From the standpoint of metabolism, methionine and cystine are probably the most important sulfur-containing compounds. We have learned that methionine is an essential amino acid and that it plays an important role in transmethylation reactions.

Sulfur is a normal constituent of the proteins of hair and feathers. During molting, hens lose appreciable amounts of sulfur. Consequently, the sulfur requirement of molting hens is high. The same is true for laying hens, since relatively large amounts of sulfur are lost in the eggs during periods of high egg production.

Only small amounts of inorganic sulfur occur in blood and tissues. The end product of sulfur metabolism is sulfuric acid, which may be neutralized by inorganic bases and excreted as inorganic sulfates, or it may be excreted in the urine as sulfate esters of phenol, indoxyl, or glucuronic acid.

Iodine. Animals and humans receiving inadequate amounts of iodine become afflicted with endemic goiter. The distribution of iodine in foods varies with soils and waters in the localities in which crops are grown.

When iodine-containing foods are digested, the iodine is carried to the thyroid gland where it eventually combines with tyrosine to form *diiodotyrosine*, which is the precursor of *thyroxine*. Both compounds are stored in the colloid of the thyroid gland. The normal iodine content of the thyroid gland is about 40 milligrams per 100 grams of gland. Thyroxine is a constitu-

ent of the thyroid hormone, which regulates the energy metabolism of the body. Work with radioactive iodine indicates that iodine is carried to the thyroid gland by the blood, it enters into organic combination at a rapid rate, and it is then transferred at an equally rapid rate to the blood stream. The use of iodized salt has become an almost universal practice, and its use is considered sound preventive medicine during pregnancy and adolescence.

Fluorine. This element is always present in small amounts in bones and teeth, but very little is known regarding its functions. It is known that the drinking of fluorine-rich waters in certain sections of the United States will cause the formation of chalky spots on the enamel of teeth. This is known as *mottling* of the enamel. On the other hand, there seems to be a relationship between adequate intake of fluorine and the incidence of dental caries (tooth decay). Tentative standards, advocated by dental authorities, call for drinking water with a content of one part per million (or more) of fluorine to control the development of dental caries.

Extensive experiments are under way to prove or disprove the hypothesis that topical treatment of children's teeth with fluorides will reduce the incidence of dental caries. Fluorides are poisonous and should be used with great care. Phosphate rock must be defluorinated before it can be used as a mineral supplement for livestock.

Iron. This element is important and must be ingested in sufficient quantity to assure normal hemoglobin formation. Iron requirements are especially high during rapid growth, in the last quarter of pregnancy, or following appreciable loss of blood. In an earlier discussion of the functions of hemoglobin, it was stated that this chromoprotein owes its oxygen-carrying ability to the presence of iron which can be alternately oxidized and reduced. Iron is essential for the formation of muscle myoglobin, and it is also a constituent of the cytochromes which are necessary for certain metabolic oxidations.

Ferrous iron is absorbed more readily than ferric iron, although both forms can be utilized. Ferric iron must be reduced to the ferrous condition in the digestive tract before it can be absorbed. Phytic acids, present in cereal grains, interfere with

iron absorption by forming insoluble iron phytates. It appears that ferrous iron is changed to *ferritin* in the intestinal mucosa. It is an interesting and somewhat puzzling fact that ferritin contains iron in the ferric state. Evidently some oxidative mechanism exists in the intestinal mucosa which changes most of the ferrous iron to ferric iron as rapidly as it is absorbed. Any ferrous iron which is not present as ferritin in the intestine and which may have escaped oxidation during absorption is converted to ferric iron in the blood stream and combined in loose combination with the serum proteins. This iron complex is not identical with ferritin. Evidently the excess of loosely combined iron in blood is changed to ferritin in the liver, where it is stored for future use. When liver reserves of ferritin are called on for blood regeneration, it is thought that the ferric iron is first reduced to the ferrous state. Most authorities express the belief that ferritin is the principal mechanism by which iron supplies are regulated and controlled for metabolic purposes.

Iron differs from other mineral elements in that it is conserved to be used again and again. A very small amount of total dietary iron is excreted. When iron is not ingested in sufficient quantities to meet the body requirements, the amount of hemoglobin in the red blood cells decreases and the number of red blood cells may also decrease. This abnormal blood condition is known as *nutritional anemia*.

Copper. This element is found in all animal tissues, but it occurs in the greatest amount in liver, kidneys, and spleen. Although copper is not a constituent of the hemoglobin molecule, it functions like an enzyme in promoting iron utilization and hemoglobin formation. The amount of copper necessary for this purpose is exceedingly small. Traces of copper are necessary for blood regeneration and growth of young animals, and its absence is associated with iron-deficiency anemias. Copper and iron deficiencies, although relatively uncommon, have been reported in domestic livestock in Australia (lambs) and in Florida (cattle).

Cobalt. Until quite recently cobalt has not been considered a dietary essential. However, nutritional anemias in livestock have been treated successfully by cobalt therapy in such widely scattered areas as Australia, New Zealand, Florida, Michigan,

and other states. The disease, which is sometimes called *pine disease*, is confined to sheep and cattle, both of which are ruminants. Little is known regarding the metabolism and function of this micronutrient. It is a normal constituent of vitamin B₁₂, which is effective in preventing pernicious anemia.

Zinc. This is another micronutrient element that seems to be essential for plant and animal nutrition. Little is known concerning its functions, although some investigators believe that it influences catalase activity. Zinc is known to be a constituent of the enzyme, carbonic anhydrase. It is probable that zinc is concerned also with the storage and utilization of the pancreatic hormone, insulin.

Manganese. This element is also found in all living tissues, although it occurs in the greatest amount in plant tissues. The simpler forms of plants fail to grow in the absence of manganese; the higher forms become chlorotic owing to a lack of chlorophyll. Animal tissues that contain appreciable amounts of manganese are liver, kidney, pancreas, and lymph node.

When animals are fed highly purified diets containing no manganese, reproductive failure occurs, indicating that this element may be essential for reproduction. It is fairly well established that manganese is essential for normal bone development in poultry and for the maintenance of hatchability of eggs. *Perosis* or *slipped-tendon disease* in chickens can be prevented or relieved by supplementing the diet with manganese salts. However, most authorities are agreed that other nutritional factors such as calcium, phosphorus, choline and biotin are also required. This poultry disease (perosis) is characterized by lameness, stiffness, and crooked legs. Little is known regarding the mechanism of manganese action, but it has been established that this element is involved in the activity of certain enzyme systems.

25 • Mineral Nutrition

From the standpoint of practical nutrition, there are eight inorganic elements of major importance. These elements can be divided into *basic* and *acidic* groups. Those possessing basic properties are *sodium*, *potassium*, *calcium*, *magnesium*, and *iron*, and *phosphorus*, *sulfur*, and *chlorine* from the acidic group. The reason for stating that these elements are of major importance lies in the fact that they are not only of primary importance in animal nutrition but they also exist in natural foods in appreciable quantities. As a result, our knowledge of the functions of these elements is much more complete than it is for the so-called "trace elements," which exist in plant and animal tissues in relatively minute amounts. It is to be expected that the mineral (ash) content of plants will vary with the type of plant, the type of soil upon which the plant is grown, climatic conditions, and the kinds and amounts of fertilizers applied to the soil.

The mineral requirements of human beings and of domestic animals can be determined by means of *balance experiments* conducted in a manner quite similar to the nitrogen balance experiments described in our discussion of protein nutrition. For example, it is possible to measure the amount of food calcium ingested during a specified experimental feeding period. It is also possible to measure the amount of calcium lost in the feces and urine during the same experimental period.

If the amount of calcium excreted *equals* the amount ingested, the person or animal is said to be in *calcium balance* or in *calcium equilibrium*. If, however, the amount of calcium excreted *exceeds* the amount ingested, it is clear that the animal is in *negative calcium balance*. Under these conditions, the skeletal reserves of calcium are being depleted. Conversely, if the

amount of calcium excreted is *less* than the amount of calcium ingested, the animal is said to be in *positive calcium balance*. Under these conditions it is evident that food calcium is being stored in the body.

We will now consider some of the quantitative aspects of mineral nutrition in order to obtain some idea of the daily requirements of man and of domestic livestock for the inorganic elements.

HUMAN FOODS AS SOURCES OF MINERALS

The following generalizations can be made regarding the mineral content of some of the more important human foods:

Calcium. Those foods that contribute appreciable amounts of calcium to the diet are milk, legumes, vegetables, and egg yolk. As a rule, the cereal seeds, such as corn, wheat, rice, and oats, are deficient in this element. Wheat flour, unless it has been enriched with calcium salts, contains even less calcium than the original wheat from which it was made, owing to the fact that the mineral elements of wheat are removed in the bran and middlings. It is for these reasons that diets consisting of bread, pastries, cereals, and meats should be supplemented with milk and vegetables.

Phosphorus. Cereals and legumes are relatively rich sources of this element. It has been stated in previous paragraphs that the mineral elements tend to concentrate in the branny seed coats. Phosphorus is present in these tissues as *phytin*, which contains phosphoric acid combined with the hexahydroxy alcohol, inositol. Other human foods rich in phosphorus are meat, eggs, and milk.

Iron. Meats, fish, and egg yolk are rich sources of iron, and peas, beans, and leafy vegetables also contain appreciable amounts. Foods that are notably poor in iron are milk, egg white, white flour, and fresh fruits.

Copper. Cereals and leafy vegetables make important contributions of copper to the human diet, and liver and fish are also relatively rich sources of this element. Milk, on the other hand, is a poor source.

Iodine. The iodine content of food plants depends on the region in which the plants are grown. Food crops grown along the sea coast usually contain appreciable amounts of iodine. Inland crops of the same types are usually deficient in this element. Sea foods are the richest in iodine of all human foods. This statement also applies to sources of iodine for livestock, since fish meals, fish-liver oils, and dried kelp are used in livestock feeding.

MINERAL REQUIREMENTS OF HUMANS

Calcium. Under normal conditions, more than 99 per cent of the body calcium is stored in the skeletal tissues. This store of skeletal calcium serves as a calcium reservoir from which calcium supplies can be doled out, as needed, for the normal functioning of the softer tissues.

Nutrition authorities are agreed that the average American dietary may be, and often is, deficient in calcium. Under these conditions it is possible for the skeletal tissues to become progressively depleted of calcium (and phosphorus), if such diets are eaten for long periods. It is only when such diets are supplemented with generous amounts of milk, legumes, and leafy vegetables that adequate calcium intakes can be ensured.

Calcium requirements are high in children, owing to increased calcium demands during rapid growth. Pregnancy and lactation call for increased calcium intakes in order to ensure adequate supplies for mother and child. Since calcium retention varies markedly in efficiency among individuals, the daily intake must always be well in excess of the calcium requirement. It is not uncommon for children to waste or lose from one-half to four-fifths of the ingested food calcium, for reasons given in our discussion of digestion and absorption.

As a result, the Food and Nutrition Board of the National Research Council have established *recommended daily allowances* for the various essential nutrients which ensure optimal nutrition by setting the nutrient levels sufficiently high to allow for "a margin of safety." Following are the recommended daily calcium allowances for human beings, as set forth by the Food and Nutrition Board: adults, 1.00 gram; pregnant women, 1.5

grams; lactating women, 2.00 grams; children to 9 years of age, 1.00 gram; children from 9 to 12 years of age, 1.2 grams; girls from 13 to 15 years of age, 1.3 grams; and boys from 13 to 15 years of age, 1.4 grams.

Phosphorus. In previous chapters, it has been pointed out that seeds, legumes, meats, and protein-rich foods are relatively rich in phosphorus. As a result, the average mixed diet should contain sufficient phosphorus to meet body requirements. For this reason, it has not been necessary to establish human allowances for this element. Since calcium and phosphorus are so closely associated in the formation of bones and teeth, it is reasonable to predict that the optimal daily intake of phosphorus by human beings should not be much less than the optimal daily intake of calcium. In other words, if the daily allowance of calcium is 1 gram, the daily phosphorus intake should be approximately 1 gram.

Iron and copper. Nutritional anemia caused by low dietary intakes of iron is characterized by low hemoglobin levels in the blood. Under ordinary conditions, with good mixed dietaries, sufficient iron is usually available for normal hemoglobin formation. Milk and white flour are notably poor sources of iron. Nutritional anemia can be produced experimentally in rats by feeding diets consisting solely of milk and white flour. This anemic condition can be prevented and cured if the diet is supplemented with iron-containing foods such as meat, cereals, and leafy vegetables. It should be borne in mind, however, that the forms of iron in foods vary in their availability.

In a previous discussion of iron metabolism, it was pointed out that iron differs from some of the other mineral elements in that it is not lost from the body so rapidly or in such large amounts as other inorganic elements. It was shown that much of the iron is conserved, stored, and used again and again in essential metabolic processes. As a result, the iron requirement of adult human beings is not high.

The following daily iron allowances have been recommended by the Food and Nutrition Board: adults, 12 milligrams; pregnant and lactating women, 15 milligrams. Daily iron allowances for children range from 6 milligrams at 1 year of age to 15 milligrams at ages from 13 to 20 years.

No allowances for copper have been established. Most authorities are agreed that sufficient copper is usually ingested in mixed diets to meet the body requirements.

Iodine. Simple goiter in human beings and domestic animals can be correlated with the iodine content of the water, the soils, and plants grown on the soils. In the United States there are regions in which simple goiter is quite prevalent. Girls at the age of puberty are more susceptible to iodine deficiencies than are other members of the population.

McClendon and co-workers have shown that regional incidence of goiter can be correlated with the iodine content of the waters in the respective regions. The following data compiled by these investigators show the relationship of iodine in water to the number of goiter cases found in four regions in the United States:

	IODINE CONTENT OF WATER, PARTS PER BILLION	GOITER CASES, PER 1000 OF POPULATION
Region I	0.010- 0.100	15-30
Region II	0.015- 1.200	5-15
Region III	0.060- 9.000	1- 5
Region IV	1.400-10.000	Less than 1

These workers also called attention to the fact that the incidence of goiter among army draftees can be correlated with the iodine content of waters and foods characteristic of the regions from which the men were drafted. Although no minimal iodine requirement has been established for human beings, McClendon has expressed the view that the daily iodine requirement is probably not less than 0.02 milligram.

The incidence of goiter is gradually decreasing, owing to the increased use of iodized salt and to improved facilities for the distribution and marketing of iodine-rich sea foods. In 1948, the Food and Nutrition Board of the National Research Council recommended that the Congress enact legislation providing that *all table salt* be fortified with iodine at a level of 0.01 per cent potassium iodide or its equivalent. To date (1950) Congress has taken no action on the recommendation.

Sulfur. Although sulfur is an essential element, there is little likelihood of sulfur deficiency. If the protein content of the

diet is adequate and sufficiently varied, sulfur requirements are easily met, without the necessity of supplementing the diet with sulfur-containing foods. Such ordinary vegetables as cabbage, broccoli, and other sulfur-containing foods make sulfur supplementation unnecessary.

Sodium, potassium, and chlorine. These essential elements are also present in adequate amounts in the average mixed human dietary. As a result, deficiencies of these elements are practically unknown. In fact, the animal body is able to adjust itself to temporary shortages of these elements to a marked degree. We have learned that the average human being usually ingests more common salt (NaCl) than the body requires. Only in cases of profuse sweating is there likely to be a need for supplementary additions of salt. Leafy vegetables and fruits contribute adequate amounts of potassium.

Other mineral elements. No standards have been established for micronutrient elements such as cobalt, manganese, fluorine, and zinc. All evidence points to the fact that these elements are usually present in adequate amounts in mixed diets containing meat, milk, eggs, vegetables, and fruits.

MINERAL REQUIREMENTS OF DOMESTIC ANIMALS

The problem of mineral nutrition of domestic livestock differs from that of human beings for a number of reasons. In the first place, domestic animals have different functional requirements. Dairy cows, for example, are required to produce milk in greater quantities than nature intended. Similarly, poultry producers are continuously breeding and feeding for higher egg production. These conditions require an ever-increasing demand for improved quality and quantity of all essential nutrients, including the essential inorganic elements.

In the second place, domestic animals differ from human beings in that they have very limited free choice, so far as quality and quantity of rations are concerned. These factors depend on the intelligence of the farmer, the availability of quality feed ingredients, and the quality of forage crops and pastures. Although it is possible to rear healthy, productive farm animals on

home-grown farm feeds, the practice of feeding mineral mixtures to livestock is increasing.

Protein concentrates of animal origin, such as tankage and fish meal, are good sources of calcium and phosphorus. The same is true of leguminous roughages. Cereals, on the other hand, are rich in phosphorus but poor in calcium. In fact, most seeds and seed by-products are lacking in calcium, and they are usually deficient in sodium and chlorine. Non-leguminous roughages vary in calcium and phosphorus, depending on the type of soil on which they are grown. As a consequence, these roughages are not considered dependable sources of these elements.

From a practical standpoint, common salt (NaCl) is of first importance. Without adequate salt supplies, cattle and sheep become unthrifty and grow slowly. Commercial feeds and farm feeds should contain from 1 to 2 per cent common salt. In addition, most successful feeders place blocks of compressed salt where it is easily accessible to the animals, for *ad libitum* feeding. Swine rations usually contain less salt than ruminant rations, since hogs do not demand more than 10 to 20 grams of salt per day. Poultry feeds usually contain up to 1 per cent of common salt.

Dairy cattle

Calcium and phosphorus. Tentative recommendations of the Committee on Animal Nutrition of the National Research Council (August 1945) are predicated on requirements for (1) growth, (2) maintenance, (3) pregnancy, and (4) lactation. Calcium and phosphorus allowances are expressed in terms of grams of each element per day per animal. For example, *growing animals* weighing 100 pounds should receive 8 grams of calcium and 6 grams of phosphorus. The daily allowances for dairy animals weighing 400 pounds are 14 grams of calcium and 11 grams of phosphorus. The highest daily allowances for growth are required when animals reach weights of 600 to 800 pounds. These animals should receive 15 grams of calcium and 12 grams of phosphorus daily. By the time animals have matured (1200 pounds live weight) daily allowances of 12 grams of each element are recommended. The same allowances are recommended for *body maintenance* of animals weighing 1200 pounds.

Requirements are higher during *pregnancy and lactation*. Allowances during the last 6 to 12 weeks of pregnancy are 22 grams of calcium and 17 grams of phosphorus. The Committee recommends that 1 gram of calcium and 0.7 gram of phosphorus be added to the ration for each pound of milk produced. These increments are in addition to the maintenance allowances.

Other mineral elements. Although no magnesium allowances have been established for dairy cattle, it is thought that the *magnesium requirement* is approximately 0.6 milligram per day per 100 pounds of body weight, when natural feeds are fed. Plant tissues and cereal seeds are relatively rich in magnesium.

In all probability the minimal *daily iodine requirement* is equivalent to 0.01 per cent potassium iodide in the ration. Iron, copper, and cobalt requirements cannot be specified with accuracy. There are regions in the United States where supplements of these elements are indicated. In all probability, daily intakes of 400 milligrams of iron, 40 milligrams of copper, and 0.1 milligram of cobalt will more than meet the average daily requirements of the dairy cow.

Beef cattle

Calcium and phosphorus. Allowances for beef cattle are expressed in terms of per cent of ration or in terms of daily intakes (grams) of calcium and phosphorus. For heifers and steers, daily growth allowances range from 20 grams of calcium and 15 grams of phosphorus at 400 pounds live weight to 15 grams of each element (daily) at 1000 pounds live weight. Daily allowances for bulls range from 24 grams of calcium and 18 grams of phosphorus at 600 pounds body weight, to 18 grams of each element when the animals weigh 1800 pounds. Daily calcium and phosphorus allowances for wintering calves and yearling cattle are 16 grams and 12 grams, respectively; daily *allowances for fattening* the same types of animals are 20 grams of each element.

Swine

Allowances are recommended for three classes of swine, namely, (1) growing and fattening pigs, (2) pregnant gilts and sows and young boars, and (3) lactating sows and breeding boars.

Calcium and phosphorus. For swine, daily allowances are expressed in terms of grams of calcium and phosphorus. Young pigs weighing 50 pounds should receive 7.4 grams of calcium and 4.9 grams of phosphorus daily. At 100 pounds live weight, daily intakes are increased to 13.7 grams of calcium and 9.1 grams of phosphorus. Calcium and phosphorus intakes should reach the maximum when growing swine reach 200 pounds live weight, with daily levels of 17.9 grams of calcium and 11.9 grams of phosphorus. No increases are recommended for heavier hogs.

Pregnant gilts and sows and young boars should receive 16.4 grams of calcium and 10.9 grams of phosphorus daily. Lactating sows and breeding boars have the highest daily requirements of all classes of swine, namely, from 27 to 41 grams of calcium and from 18 to 27 grams of phosphorus.

Other mineral nutrients. No allowances have been recommended for other essential inorganic nutrients. It is common knowledge that goitrous and hairless pigs are produced in relatively large numbers in iodine-deficient regions. To prevent this situation, a stabilized form of iodine must be incorporated in the diet. Experienced swine feeders recommend 0.1 milligram of iodine per day per animal for pigs of medium weight and 0.2 milligram per 100 pounds body weight per day for pregnant sows. Suckling pigs should receive not less than 15 milligrams of iron per day, since this is the age when anemia occurs and when pig mortality is high. This amount of iron will usually maintain hemoglobin at birth levels. No requirements have been established for magnesium, manganese, and cobalt, although these elements are considered essential for swine.

Poultry

Mineral allowances for poultry are expressed (1) in terms of percentage composition of the ration or (2) in terms of weight of nutrient per pound of feed.

Tentative National Research Council recommendations call for starting chick rations with a calcium content of 1 per cent. Rations of laying and breeding hens should contain 2.25 per cent of this element. For the latter group, all the calcium need not be incorporated in the mixed feed, since oyster shell and other calcium-containing materials may be fed "free choice."

The phosphorus content of a starting chick ration should not be less than 0.6 per cent, and at least one-third of the total phosphorus should be in the form of non-phytin phosphorus, to ensure availability. Rations for both classes of poultry should contain 0.5 per cent common salt. On account of the fact that manganese is thought to be one of the nutritive factors that prevents perosis (slipped-tendon disease) in poultry, this element should be added at rates of 25 milligrams per pound of starting chick rations and 15 milligrams per pound in rations of laying and breeding hens. Rations for both classes of poultry should contain 0.5 milligram of iodine per pound of feed.

Tentative calcium allowances for turkeys range from 2.0 per cent (in the ration) for poults to 2.25 per cent for breeders. Phosphorus allowances for the same types of birds range from 1.0 to 0.75 per cent. Manganese allowances per pound of feed are as follows: poults, 25 milligrams; and breeders, 15 milligrams.

It should be borne in mind that all recommended allowances mentioned in this chapter represent optimal amounts in order to ensure adequate supplies of all essential nutrients. For more detailed information regarding the recommendations of the Food and Nutrition Board and the Committee on Animal Nutrition of the National Research Council, the reader is referred to the tables in the Appendix.

Appendix

Boys,										
13-15 yr (108 lb, 49 kg)	3200	85	1.4	15	5000	1.5	2.0	15	90	400
16-20 yr (141 lb, 64 kg)	3800	100	1.4	15	6000	1.7	2.5	17	100	400

¹ Objectives toward which to aim in planning practical dietaries. The recommended allowances can be attained with a good variety of common foods which will also provide other minerals and vitamins for which requirements are less well known.

² Calorie allowances must be adjusted up or down to meet specific needs. The calorie values in the table are therefore not applicable to all individuals but rather represent group averages. The proper calorie allowance is that which over an extended period will maintain body weight or rate of growth at the level most conducive to well-being.

³ The allowance depends on the relative amounts of vitamin A and carotene. The allowances of the table are based on the premise that approximately two-thirds of the vitamin A value of the average diet in this country is contributed by carotene and that carotene has half or less than half the value of vitamin A.

⁴ For adults (except pregnant and lactating women) receiving diets supplying 2000 calories or less, such as reducing diets, the allowances of thiamine and niacin may be 1 milligram and 10 milligrams, respectively. The fact that figures are given for different calorie levels for thiamine and niacin does not imply that we can estimate the requirement of these factors within 500 calories, but they are added merely for simplicity of calculation. In the present revision, riboflavin allowances are based on body weight rather than caloric levels. Other members of the B complex also are required, though no values can be given. Foods supplying adequate thiamine, riboflavin, and niacin will tend to supply sufficient of the remaining B vitamins.

⁵ There is evidence that the male adult needs relatively little iron. The need will usually be provided for if the diet is satisfactory in other respects.

⁶ The need for supplemental vitamin D by vigorous adults leading a normal life seems to be minimum. For persons working at night and for nuns and others whose habits shield them from the sunlight, as well as for elderly persons, the ingestion of small amounts of vitamin D is desirable.

⁷ During the latter part of pregnancy the calorie allowance should increase to approximately 20 per cent above the preceding level. The value of 2400 calories represents the allowance for pregnant, sedentary women.

⁸ Allowances for children are based on the needs for the middle year in each group (as 2, 5, 8, etc.) and are for moderate activity and for average weight at the middle year of the age group.

⁹ Needs for infants increase from month to month with size and activity. The amounts given are for approximately 6 to 8 months. The dietary requirements for some of the nutrients such as protein and calcium are less if derived largely from human milk.

* *Recommended Dietary Allowances (Human)*. National Research Council Reprint and Circular Series, No. 129. Food and Nutrition Board, National Research Council, Washington, D. C. Revised October 1948.

RECOMMENDED NUTRIENT ALLOWANCES FOR BEEF CATTLE *
(All feeds or rations are calculated on the basis of 90 per cent dry matter)

Body Weight, lb	Expected Daily Gain, lb	Daily Feed		Digestible Nutrients		Digestible Protein		Calcium		Phosphorus		Carotene 1 Daily per Animal, mg
		Per Cent of Live Weight	Per Animal, lb	Per Cent in Ration	Daily per Animal, lb	Per Cent in Ration	Daily per Animal, lb	Per Cent in Ration	Daily per Animal, gm	Per Cent in Ration	Daily per Animal, gm	
Heifers and Steers, Normal Growth												
400	1.6	3.0	12	58	7.0	0.9	0.37	20	0.28	15	25	
600	1.4	2.7	16	53	8.5	0.9	0.25	18	0.21	15	35	
800	1.2	2.4	19	50	9.5	0.9	0.19	16	0.17	15	45	
1000	1.0	2.1	21	50	10.5	0.9	0.16	15	0.16	15	55	
Bulls, Growth and Maintenance (Moderate Activity)												
600	2.3	2.7	16	63	10.0	1.3	0.33	24	0.25	18	35	
800	1.7	2.3	18	61	11.0	1.4	0.28	23	0.22	18	45	
1000	1.6	2.2	22	55	12.0	1.4	0.22	22	0.18	18	55	
1200	1.4	2.0	24	54	13.0	1.4	0.19	21	0.17	18	65	
1400	1.0	1.9	26	54	14.0	1.4	0.17	20	0.15	18	75	
1600	...	1.7	26	54	14.0	1.4	0.15	18	0.15	18	90	
1800	...	1.6	26	54	14.0	1.4	0.15	18	0.15	18	100	
Wintering Weanling Calves												
400	1.0	2.8	11	55	6.0	0.7	0.32	16	0.24	12	25	
500	1.0	2.6	13	54	7.0	0.8	0.27	16	0.20	12	30	
600	1.0	2.5	15	53	8.0	0.8	0.24	16	0.18	12	35	
Wintering Yearling Cattle												
600	1.0	2.7	16	50	8.0	0.8	0.22	16	0.17	12	35	
700	1.0	2.4	17	50	8.5	0.8	0.21	16	0.16	12	40	
800	0.7	2.3	18	50	9.0	0.8	0.20	16	0.15	12	45	
900	0.5	2.0	18	50	9.0	0.8	0.20	16	0.15	12	50	
Wintering Pregnant Heifers (Weights are for Beginning of Winter Period; Gains, Average for Period)												
700	1.5	2.9	20	50	10.0	0.9	0.20	18	0.18	16	55	
800	1.3	2.3	20	50	10.0	0.9	0.20	18	0.18	16	55	
900	0.8	2.0	18	50	9.0	0.8	0.20	16	0.18	15	55	
1000	0.5	1.8	18	50	9.0	0.8	0.20	16	0.18	15	55	

Wintering Mature Pregnant Cows (Weights are for Beginning of Winter Period; Gains, Average for Period)

800	1.5	2.8	22	50	11.0	4.5	1.0	0.20	22	0.18	18	55
900	1.0	2.2	20	50	10.0	4.5	0.9	0.20	18	0.18	16	55
1000	0.4	1.8	18	50	9.0	4.5	0.9	0.20	16	0.18	15	55
1100	0.2	1.6	18	50	9.0	4.5	0.8	0.20	16	0.18	15	60
1200	0.0	1.5	18	50	9.0	4.5	0.8	0.20	16	0.18	15	65

Cows Nursing Calves, First 3 to 4 Months After Parturition

900-1100	None	...	28	50	14.0	5.0	1.4	0.24	30	0.18	24	300
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Fattening Calves Finished as Short Yearlings

400	Average	3.0	12	67	8.0	9.2	1.1	0.37	20	0.28	15	25
500	for period,	2.8	14	68	9.5	8.6	1.2	0.31	20	0.25	16	30
600	2.0	2.7	16	68	11.0	8.1	1.3	0.28	20	0.23	17	35
700		2.6	18	68	12.0	7.8	1.4	0.25	20	0.22	18	40
800	pounds	2.5	20	68	13.5	7.5	1.5	0.22	20	0.20	18	45
900	daily	2.3	21	68	14.5	7.2	1.5	0.21	20	0.19	18	50

Fattening Yearling Cattle

600	Average	3.0	18	65	11.5	7.2	1.3	0.25	20	0.21	17	35
700	for period,	3.0	21	65	13.5	7.0	1.4	0.21	20	0.19	18	40
800	2.2	2.8	22	65	14.0	6.8	1.5	0.20	20	0.19	19	45
900		2.7	24	65	15.5	6.7	1.6	0.18	20	0.18	20	50
1000	pounds	2.6	26	65	17.0	6.5	1.7	0.17	20	0.17	20	55
1100	daily	2.4	27	65	17.5	6.3	1.7	0.16	20	0.16	20	60

Fattening 2-Year-Old Cattle

800	Average	3.0	24	62	15.0	6.3	1.5	0.18	20	0.18	20	45
900	for pe-	2.9	26	62	16.0	6.3	1.6	0.17	20	0.17	20	50
1000	riod, 2.4	2.7	27	62	17.0	6.3	1.7	0.16	20	0.16	20	55
1100	pounds	2.6	29	62	18.0	6.3	1.8	0.15	20	0.15	20	60
1200	daily	2.4	29	62	18.0	6.3	1.8	0.15	20	0.15	20	65

¹ The recommended carotene allowances for fattening animals is at the same rate as for cattle in other classifications because this is about the minimum rate that will result in significant storage and thus assure contribution of vitamin A value for human use from the beef liver and fat. For optimum growth or feed-lot gains and freedom from clinical symptoms, 1.5 milligrams carotene for each 100 pounds body weight suffices for cattle previously depleted of body stores, and this level may be used except for pregnant or lactating cows when economically necessary. The vitamin A value of the liver and the body fat of animals so fed, however, would be practically nil. Actually, no dietary carotene or vitamin A is needed so long as the animals have sufficient storage reserve to meet physiological needs.

* Recommended Nutrient Allowances for Beef Cattle (No. IV). Committee on Animal Nutrition, National Research Council, Washington, D. C. September 1945.

RECOMMENDED NUTRIENT ALLOWANCES FOR DAIRY CATTLE *

(Tentative)

Weight of Animal, lb	Expected Gain, lb		Daily Allowance per Animal ¹					
	Jersey	Holstein	Digestible Protein, lb	Total Digestible Nutrients, lb	Calcium, gm	Phosphorus, gm	Carotene, mg	Vitamin D, I.U.
Growth								
50	0.5	...	0.30	1.0	4	3	2	150
100	1.0	0.8	0.45	2.0	8	6	6	300
150	1.3	1.4	0.60	3.0	12	8	10	450
200	1.4	1.6	0.70	4.0	13	9	12	600
400	1.2	1.8	0.80	6.5	14	11	25	1200
600	0.8	1.4	0.85	8.5	15	12	35
800	1.1	1.2	0.90	10.0	15	12	45	³
1000	...	1.3	0.95	11.0	14	12	60
1200	...	1.2	1.00	12.0	12	12	70
Maintenance ⁴								
700			0.45	6.0	7	7	40	³
1000			0.60	8.0	10	10	60
1200			0.70	9.5	12	12	70
1400			0.80	11.0	14	14	80
Pregnancy (per 1000 lb) (last 6 to 12 weeks)			1.2	14.0	22	17	90	³
Lactation (per pound milk)								
3.0% fat			0.040	0.28	1	0.7	5	5
4.0% fat			0.045	0.32	1	0.7
5.0% fat			0.050	0.37	1	0.7
6.0% fat			0.055	0.42	1	0.7

¹ Thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, and vitamin K are synthesized by bacteria in the rumen, and it appears that adequate amounts of these vitamins are furnished by a combination of rumen synthesis and natural feedstuffs. Manganese, iron, copper, and cobalt are clearly essential, but the amounts needed are not known. For growth 0.6 gram magnesium is needed per 100 pounds body weight.

² Calves should receive colostrum the first few days after birth, as a source of vitamin A and other essential factors.

³ Although vitamin D is known to be required, the data are inadequate to warrant specific figures for older growing animals and for maintenance, reproduction, and lactation.

⁴ When calculating the allowances for lactating heifers that are still growing, it is recommended that the figure for growth rather than maintenance be used.

⁵ When adequate amounts of vitamins A and D are fed for normal reproduction, extra amounts will probably not stimulate milk production but will increase the vitamin content of the milk.

* *Recommended Nutrient Allowances for Dairy Cattle (No. III)*. Committee on Animal Nutrition, National Research Council, Washington, D. C. August 1945.

Live Weight, lb	Expected Daily Gain or Loss, lb	Total Feed (Air-Dry Basis), lb	Total Digestible Protein		Total Digestible Nutrients		Calcium Intake		Phosphorus Intake		Salt, lb	Carotene Intake, mg
			In Ration, %	Per Sheep, lb	In Ration, %	Per Sheep, lb	In Ration, gm	90% Dry Matter, %	In Ration, gm	90% Dry Matter, %		
Bred Ewes, First 100 Days of Gestation												
100	0.12	3.5	5.0	0.17	50	1.7	3.2	0.20	2.5	0.16	0.03	6.0
110	0.12	3.6	5.0	0.18	50	1.8	3.2	0.20	2.6	0.16	0.03	6.6
120	0.12	3.7	5.0	0.19	50	1.9	3.3	0.20	2.7	0.16	0.03	7.2
130	0.12	3.8	5.0	0.20	50	2.0	3.4	0.20	2.7	0.16	0.03	7.8
Bred Ewes, Last 6 Weeks before Lambing												
110	0.25	4.0	5.0	0.21	53	2.1	4.3	0.24	3.2	0.18	0.03	6.6
120	0.25	4.1	5.0	0.22	54	2.2	4.4	0.24	3.3	0.18	0.03	7.2
130	0.25	4.2	5.0	0.23	54	2.3	4.5	0.24	3.4	0.18	0.03	7.8
140	0.25	4.3	5.0	0.24	55	2.4	4.7	0.24	3.5	0.18	0.03	8.4
150	0.25	4.4	5.0	0.25	55	2.5	4.8	0.24	3.6	0.18	0.03	9.0
Ewes in Lactation												
100	-0.10	4.5	6.0	0.27	58	2.5	6.1	0.30	4.5	0.22	0.03	6.0
110	-0.10	4.6	6.0	0.28	58	2.6	6.2	0.30	4.6	0.22	0.03	7.1
120	-0.10	4.7	6.0	0.28	58	2.7	6.4	0.30	4.7	0.22	0.03	7.8
130	-0.10	4.8	6.2	0.30	58	2.8	6.5	0.30	4.8	0.22	0.03	8.4
140	-0.10	4.9	6.1	0.30	58	2.9	6.6	0.30	4.9	0.22	0.03	9.1
150	-0.10	5.0	6.1	0.31	58	3.0	6.8	0.30	5.0	0.22	0.03	9.7
Ewes—Lambs and Yearlings												
70	0.35	3.0	7.3	0.22	58	1.8	3.0	0.27	2.7	0.18	0.02	3.8
90	0.30	3.2	6.9	0.22	58	1.9	3.0	0.27	2.7	0.18	0.02	5.0
110	0.20	3.5	5.7	0.20	54	1.9	3.2	0.24	2.8	0.16	0.03	6.0
130	0.10	3.8	5.3	0.20	54	2.0	3.1	0.24	2.7	0.16	0.03	7.1
Rams—Lambs and Yearlings												
75	0.45	3.5	6.8	0.24	58	2.1	3.8	0.27	3.2	0.18	0.02	4.1
100	0.40	4.0	6.0	0.24	60	2.3	4.0	0.27	3.4	0.18	0.03	5.5
125	0.35	4.0	6.0	0.24	60	2.4	3.6	0.27	3.3	0.18	0.03	6.9
150	0.30	4.3	5.3	0.23	60	2.6	3.7	0.24	3.3	0.16	0.03	8.2
175	0.20	4.5	5.1	0.23	58	2.6	3.7	0.24	3.3	0.16	0.03	9.6
Fattening Lambs												
50	0.25	2.1	8.1	0.17	57	1.2	2.5	0.30	2.1	0.20	0.02	3.0
60	0.30	2.3	7.8	0.18	60	1.4	2.6	0.30	2.2	0.20	0.02	3.6
70	0.35	2.7	7.0	0.19	63	1.7	2.9	0.27	2.4	0.18	0.02	4.2
80	0.35	2.9	6.9	0.20	65	1.9	2.9	0.27	2.4	0.18	0.02	4.2
90	0.25	3.0	6.7	0.20	66	2.0	2.7	0.27	2.3	0.18	0.02	5.4

* Recommended Nutrient Allowances for Sheep (No. V). Committee on Animal Nutrition, National Research Council, Washington, D. C. August 1949.

* Recommended Nutrient Allowances for Sheep (No. V). Committee on Animal Nutrition, National Research Council, Washington, D. C. August 1949.

DAILY PROTEIN AND ENERGY ALLOWANCES FOR GROWING HORSES *
(Mature weight in pounds)

Lb.	600					800				
	Age, months	Daily Gain, lb	Dry Mat- ter, lb	Digest- ible Pro- tein, lb	Total Digest- ible Nu- trients, lb	Age, months	Daily Gain, lb	Dry Mat- ter, lb	Digest- ible Pro- tein, lb	Total Digest- ible Nu- trients, lb
200	3.5	1.1	6.7	0.77	4.2	2.6	1.4	7.2	0.93	4.5
400	14.0	0.6	9.1	0.57	5.7	8.0	0.9	9.9	0.80	6.2
600	42.0	0.0	10.2	0.45	6.4	19.0	0.5	11.7	0.65	7.3
800	44.0	0.0	12.6	0.54	7.9
1000
1000						1200				
200	2.0	1.6	7.5	1.04	4.7	1.9	1.9	7.7	1.06	4.8
400	6.0	1.2	10.2	0.94	6.4	5.2	1.6	10.4	1.03	6.5
600	14.0	0.8	12.3	0.77	7.7	10.0	1.2	13.3	0.93	8.3
800	24.0	0.5	13.8	0.74	8.6	17.0	0.8	14.7	0.87	9.2
1000	44.0	0.0	15.0	0.64	9.4	25.0	0.4	16.2	0.85	10.1
1200	45.0	0.0	17.1	0.72	10.7
1400						1600				
200	1.2	2.5	8.2	1.22	5.1	0.9	3.3	8.5	1.28	5.3
400	4.0	2.1	11.0	1.16	6.9	3.0	2.8	11.8	1.43	7.4
600	7.5	1.6	13.4	1.13	8.4	5.5	2.4	13.9	1.33	8.7
800	12.0	1.3	15.8	1.05	9.9	9.0	1.8	16.6	1.23	10.4
1000	19.5	0.8	17.0	0.97	10.6	13.0	1.2	18.4	1.17	11.5
1200	26.0	0.4	18.4	0.95	11.5	19.0	1.0	19.5	1.11	12.2
1400	45.0	0.0	19.4	0.81	12.1	26.0	0.5	20.6	1.06	12.9
1600	48.0	0.0	21.1	0.86	13.2
1800										
200	0.6	4.0	9.0	1.39	5.6					
400	2.4	3.8	12.3	1.50	7.7					
600	4.5	3.3	14.4	1.49	9.0					
800	7.0	2.7	17.0	1.44	10.6					
1000	9.6	2.1	19.5	1.40	12.2					
1200	13.2	1.6	20.8	1.34	13.0					
1400	18.4	1.0	22.1	1.26	13.8					
1600	26.4	0.5	22.7	1.18	14.2					
1800	48.0	0.0	23.2	0.95	14.5					

* *Recommended Nutrient Allowances for Horses (No. VI).* Committee on Animal Nutrition, National Research Council, Washington, D. C. March 1949.

Description of Pigs			Energy and Protein		Inorganic Nutrients				Vitamins								
Class	Live Weight, lb	Ex-pected Daily Gain, lb	Total Feed (Air-Dry Basis), lb	Equiv-alent Total Digest-ible Nu-trients, lb	Crude Protein, lb	Cal-cium, gm	Phos-phorus, gm	Sod-ium, gm	Po-tas-sium, gm	Caro-tene, mg	Vita-min A, I.U.	Vita-min D, I.U.	Thia-mine, mg	Ribo-flavin, mg	Nia-cin, mg	Panto-thenic Acid, mg	Pyri-dox-ine, mg
Growing, fattening pigs	50	0.90	2.7	2.0	0.6	7.4	4.9	2.7	1.3	2.0	1,300	135	1.4	2.1	7.0	10.0	1.6
	100	1.50	5.0	3.8	0.8	13.7	9.1	5.0	2.5	4.0	2,600	250	2.5	3.8	12.5	18.5	3.0
	150	1.75	6.6	5.0	0.9	15.8	10.5	6.6	3.8	6.0	3,900	330	3.3	5.0	16.5
	200	1.80	7.5	5.6	1.0	17.9	11.9	7.5	5.0	8.0	5,200	375	3.8	5.7	19.0
	250	1.80	8.3	6.2	1.0	17.9	11.9	8.3	6.0	10.0	6,500	415	4.2	6.3	21.0
Pregnant gilts and sows; young boars	...	0.75 (min)	6.0	4.5	0.9	16.4	10.9	6.0	6.0	20.0	13,000	300	3.0
	10-15	7.5-11.3	1.5-2.3	27-41	18-27	12.5	12.5	40.0	26,000	625	6.3
Lactating sows; breeding boars																	

Notes:

Vitamin D: This requirement may be fully met by ultraviolet radiation from the sun.
Thiamine: Amounts specified will permit development of a normal pig but will not provide for thiamine storage.
Vitamin E: Required, but amounts unknown.
Iron: Requirements beyond weaning unknown. For suckling pigs 15 milligrams iron daily for first 3 weeks will maintain birth hemoglobin level.
Copper: Requirements beyond weaning unknown. For suckling pigs 15 milligrams iron daily for first 3 weeks will maintain birth hemoglobin level.
Copper: Usually taken as 5 per cent of the iron administered.
Cobalt: For sheep, 4 ounces CuSO₄ per ton NaCl has been enough to relieve deficiency symptoms. Swine requirements unknown.
Iodine: For pregnant sows, 0.2 milligram iodine per 100 pounds body weight has been proposed. Requirements for other swine are probably somewhat less.
Magnesium: } Required, but amounts unknown.
Manganese: }
Zinc:

* Recommended Nutrient Allowances for Swine (No. II). Committee on Animal Nutrition, National Research Council, Washington, D. C. August 1944.

RECOMMENDED NUTRIENT ALLOWANCES FOR CHICKENS *

	Total Protein, %	Vitamins								Minerals					
		Vitamin A Activity, ¹ I.U./lb	Vitamin D, A.O.A.C. units/lb	Thiamine, mg/lb	Riboflavin, mg/lb	Pantothenic Acid, mg/lb	Nicotinic Acid, mg/lb	Pyridoxine, mg/lb	Biotin, mg/lb	Choline, mg/lb	Calcium, %	Phosphorus, ² %	Salt, ³ %	Manganese, mg/lb	Iodine, ⁴ mg/lb
Starting chicks, 0-8 weeks	20	2000	180	0.9	1.6	5.0	8.0	1.6	0.045	700	1.00	0.60	0.5	25	0.5
Growing chicks, 8-18 weeks	16	2000	180	?	0.9	?	?	?	?	?	1.00	0.60	0.5	?	0.5
Laying hens	15	3300	450	?	0.9	2.5	?	1.6	?	?	2.25 ⁵	0.75	0.5	?	0.5
Breeding hens	15	3300	450	?	1.3	5.0	?	1.6	0.070	?	2.25 ⁵	0.75	0.5	15	0.5

¹ May be fish oil vitamin A or provitamin A from vegetable sources.

² Inorganic phosphorus should constitute 0.2 per cent of the total feed.

³ This figure represents added salt or sodium chloride.

⁴ This allowance is larger than that specified in National Research Council Reprint and Circular Series No. 111, May, 1942. *Iodine—Its Necessity and Stabilization*, because of changes in the feed supply which increase the need for iodine.

⁵ This amount of calcium need not be incorporated in the mixed feed inasmuch as calcium supplements fed free choice are considered as part of the ration.

* *Recommended Nutrient Allowances for Poultry (No. I)*. Committee on Animal Nutrition, National Research Council, Washington, D. C. June 1944.

RECOMMENDED NUTRIENT ALLOWANCES FOR TURKEYS *

	Total Protein, %	Vitamins				Minerals			
		Vitamin A Activity, ¹ I.U./lb	Vitamin D, ² A.O.A.C. units/lb	Riboflavin, mg/lb	Choline, mg/lb	Calcium, %	Phosphorus, ³ %	Salt, ⁴ %	Manganese, mg/lb
Starting poults, 0-8 weeks	24	4000	800	2.0	900	2.00	1.00	0.5	25
Growing turkeys, 8-16 weeks	20 ⁵	4000	800	?	?	2.00	1.00	0.5	?
Turkey breeders	15	4000	800	1.6	?	2.25 ⁶	0.75	0.5	15

¹ May be either fish oil vitamin A or provitamin A from vegetable sources.

² This allowance should prove adequate for vitamin D from either fish oil or irradiated animal sterols when the ration contains the recommended allowances for calcium and phosphorus and when the minimum amount of inorganic phosphorus suggested in footnote 3 is present in the ration. If the ration contains materially less calcium and phosphorus, it is necessary to increase the amount of vitamin D when it is obtained from fish oil.

³ Inorganic phosphorus should constitute 0.4 per cent of the total feed.

⁴ This figure represents added salt or sodium chloride.

⁵ The protein content of rations for growing turkeys from 16 weeks to market weight may be reduced to 16 per cent.

⁶ This amount of calcium need not be incorporated in the mixed feed inasmuch as calcium supplements fed free choice are considered as part of the ration.

* *Recommended Nutrient Allowances for Poultry (No. I)*. Committee on Animal Nutrition, National Research Council, Washington, D. C. June 1944.

CHEMICAL COMPOSITION OF SELECTED HUMAN FOODS

(Nutritive value of 100 grams, edible portion)

Food Item	Water, %	Food Energy, Cal	Pro- tein, gm	Fat, gm	Carbo- hy- drate, gm	Calcium, mg	Phos- phorus, mg	Iron, mg	Vitamin A Value, I.U.	Thiamine, mg	Ribo- flavin, mg	Niacin, mg	Ascorbic Acid, mg
Milk, Cream, Ice Cream, Cheese													
Milk													
Dry whole	3.5	496	25.8	26.7	38.0	949	728	0.58	1400	0.30	1.46	0.7	6
evaporated, unsweetened	73.7	139	7.0	7.9	9.9	243	195	0.17	400	0.05	0.36	0.2	1
Fresh skim	90.5	35	3.5	0.1	5.1	118	93	0.07	Trace	0.04	0.18	0.1	1
Fresh whole	87.0	69	3.5	3.9	4.9	118	93	0.07	160	0.04	0.17	0.1	1
Cream, ice cream													
Cream (20 per cent), sweet or sour	72.5	208	2.9	20.0	4.0	97	77	0.06	830	0.03	0.14	0.1	1
Ice cream, plain	62.0	210	4.0	12.3	20.8	132	104	0.10	540	0.04	0.19	0.1	Trace
Cheese													
Cheddar type	39	393	23.9	32.3	1.7	873	610	0.57	1740	0.04	0.50	0.2	0
Cottage	74.0	101	19.2	0.8	4.3	82	263	0.46	30	0.02	0.29	0.1	0
Fats, Oils													
Bacon, medium fat	20	626	9.1	65	1.1	13	108	0.8	0	0.42	0.10	2.1	0
Butter	15.5	733	0.6	81	0.4	16	16	0.2	3300	Trace	0.01	0.1	0
Lard, other shortening	0	900	0	100	0	0	0	0	0	0	0	0	0
Margarine with vitamin A added	15.5	733	0.6	81	0.4	2	15	0.2	1980	0	0	0	0
Salt pork, fat	8	781	3.9	85	0	2	42	0.6	0	0.18	0.04	0.9	0
Eggs													
Whole, dried	2	593	48.2	43.3	2.6	187	800	8.7	4460	0.35	1.23	0.2	0
Whole, fresh	74.0	158	12.8	11.5	0.7	54	210	2.7	1140	0.12	0.34	0.1	0
Meat, Poultry, Fish													
Beef													
Loin steaks (wholesale loin)	57	293	16.9	25	0	10	182	2.5	0	0.10	0.13	4.6	0
Round steak (wholesale round)	67	194	19.3	13	0	11	208	2.9	0	0.12	0.15	5.2	0
Lamb													
Leg roast (wholesale leg)	63.7	230	18.0	17.5	0	10	194	2.7	0	0.21	0.26	5.9	0
Sirloin chop (wholesale leg)	63.7	230	18.0	17.5	0	10	194	2.7	0	0.21	0.26	5.9	0
Pork													
Ham, fresh	53	340	15.2	31	0	9	164	2.3	0	0.96	0.19	4.1	0
Ham, smoked	42	384	16.9	35	0.3	10	182	2.5	0	0.78	0.19	3.8	0
Pork links, sausage	41.9	446	10.8	44.8	0	6	116	1.6	0	0.22	0.15	2.3	0
Poultry													
Chicken, roasters	66.0	194	20.2	12.6	0	16	218	1.9	Trace	0.11	0.18	8.6	..
Turkey, medium fat	58.3	262	20.1	20.2	0	23	320	3.8	Trace	0.12	0.19	7.9	..
Fish and shellfish													
Cod	82.6	70	16.5	0.4	0	18	189	0.9	0.04	0.05	2.3	2
Salmon, canned	67.4	169	20.6	9.6	0	67	286	1.3	80	0.03	0.18	6.5	0

Dry beans and peas	71.0	117	5.7	2.0	19.0	49	154	3.4	70	0.05	0.05	0.8	4
Beans, canned, baked	12.6	341	20.7	1.3	61.6	68	381	7.5	0	0.60	0.24	2.1	2
Beans, lima, dry seed	10.0	354	24.5	1.0	61.7	73	397	6.0	370	0.87	0.29	3.0	2
Peas, split													
Nuts													
Peanut butter	1.7	619	26.1	47.8	21.0	74	393	1.9	0	0.20	0.16	16.2	0
Peanuts, roasted	2.6	600	26.9	44.2	23.6	74	393	1.9	0	0.30	0.16	16.2	0
Fresh Vegetables													
Asparagus	93.0	26	2.2	0.2	3.9	21	62	0.9	1000	0.16	0.17	1.2	33
Beans, snap	88.9	42	2.4	0.2	7.7	65	44	1.1	630	0.08	0.10	0.6	19
Beets	87.6	46	1.6	0.1	9.6	27	43	1.0	20	0.03	0.05	0.4	10
Carrots	88.2	45	1.2	0.3	9.3	39	37	0.8	12000	0.07	0.06	0.5	6
Corn, sweet, white, or yellow	73.9	108	3.7	1.2	20.5	9	120	0.5	390	0.15	0.14	1.4	12
Cucumbers	96.1	14	.7	0.1	2.7	10	21	0.3	0	0.04	0.09	0.2	8
Lettuce, headed	94.8	18	1.2	0.2	2.9	22	25	0.5	540	0.06	0.07	0.2	8
Onions, mature	87.5	49	1.4	0.2	10.3	32	44	0.5	50	0.03	0.02	0.1	9
Peas, green	74.3	101	6.7	0.4	17.7	22	122	1.9	680	0.36	0.18	2.1	26
Potatoes	77.8	85	2.0	0.1	19.1	11	56	0.7	20	0.11	0.04	1.2	17
Spinach	92.7	25	2.3	0.3	3.2	...	55	3.0	9420	0.12	0.24	0.7	59
Sweet potatoes	68.5	125	1.8	0.7	27.9	30	49	0.7	7700	0.10	0.06	0.7	22
Tomatoes	94.1	23	1.0	0.3	4.0	11	27	0.6	1100	0.06	0.04	0.6	23
Turnips	90.9	35	1.1	0.2	7.1	40	34	0.5	Trace	0.06	0.06	0.5	28
Fresh Fruit													
Apples	84.1	64	0.3	0.4	14.9	6	10	0.3	90	0.04	0.02	0.2	5
Bananas	74.8	99	1.2	0.2	23	8	28	0.6	430	0.09	0.06	0.6	10
Strawberries	90.0	41	0.8	0.6	8.1	28	27	0.8	60	0.03	0.07	0.3	60
Grapefruit	88.8	44	0.5	0.2	10.1	17	18	0.3	Trace	0.04	0.02	0.2	40
Lemons	89.3	44	0.9	0.6	8.7	14	10	0.1	0	0.04	Trace	0.1	45
Oranges	87.2	50	0.9	0.2	11.2	33	23	0.4	190	0.08	0.03	0.2	49
Peaches	86.9	51	0.5	0.1	12.0	8	22	0.6	880	0.02	0.05	0.9	8
Rhubarb	94.9	18	0.5	0.1	3.8	...	25	0.5	30	0.01	...	0.1	9
Grain Products													
Flour													
Wheat, patent	12	355	10.8	0.9	75.9	19	93	0.7	0	0.07	0.03	0.8	0
Wheat, patent, enriched	12	355	10.8	0.9	75.9	19	93	2.9	0	0.44	0.26	3.5	0
Whole wheat	11	360	13.0	2.0	72.4	38	385	3.8	0	0.56	0.12	5.6	0
Breakfast cereals													
Corn flakes	9.3	359	7.9	0.7	80.3	10	56	1.0	0	0.16	0.08	1.6	0
Oatmeal	8.3	396	14.2	7.4	68.2	54	365	5.2	0	0.55	0.14	1.1	0
Shredded wheat	7.7	369	10.4	1.4	78.7	38	385	3.8	0	0.20	0.14	4.2	0
Other cereals													
Hominy	11.4	357	8.5	0.8	78.9	11	70	1.0	0	0.15	0.05	0.9	0
Macaroni, spaghetti	11	360	13	1.4	73.9	22	144	1.2	0	0.13	0.08	2.1	0
Sugars, Sweets													
Honey	20	319	0.3	0	79.5	5	16	0.9	0	Trace	0.04	0.2	4
Sugar, granulated or powdered	0.5	398	0	0	99.5	0	0	0.1	0	0	0	0	0
Miscellaneous													
Cocoa	4.3	329	9.0	18.8	31.0	...	709	2.7	0	Trace	0.39	2.3	0
Yeast, dried, brewers'	7.0	348	46.1	1.6	37.4	106	1893	18.2	0	9.69	5.45	36.2	0

CHEMICAL COMPOSITION OF SELECTED RUMINANT FEEDS

	Dry Matter, %	Crude Protein, %	Total Digestible Nutrients, %	Digestible Protein, %	Calcium		Phosphorus		Carotene, mg/lb
					%	gm/lb	%	gm/lb	
Air-dry forages	92.8	15.4	50.4	10.8	1.51	6.85	0.21	0.95	19.4
Alfalfa hay	93.4	16.9	53.0	12.5	1.56	7.08	0.22	1.00	44.6
Alfalfa meal, dehydrated	87.1	13.6	49.6	9.7	1.01	4.58	0.14	0.64	11.0
Clover hay, red	89.3	5.8	48.6	1.9	0.45	2.04	0.10	0.45
Corn stover, very dry	88.0	12.3	45.5	5.4	0.97	4.40	0.23	1.04	22.4
Lespedeza hay	91.9	4.4	45.5	1.5	0.23	1.04	0.20	0.91	0.1
Oat straw	90.4	5.7	49.2	2.6	0.50	2.27	0.10	0.45	11.5
Prairie hay, moderately green	90.8	14.8	50.6	11.1	1.26	5.72	0.22	1.00
Soybean hay	88.7	6.2	46.9	2.9	0.31	1.41	0.13	0.59	9.2
Timothy hay									
Silages, roots, tubers									
Alfalfa silage, slightly wilted	31.1	5.7	18.8	4.0	0.38	1.72	0.06	0.27	14.9
Corn silage, well matured	29.1	2.4	19.0	1.0	0.08	0.36	0.08	0.36	4.0
Potatoes	21.1	2.1	17.0	0.7	0.01	0.05	0.06	0.27
Grains, seeds, and by-product concentrates									
Barley	90.4	12.8	77.1	10.0	0.07	0.32	0.32	1.45
Brewers' grains, dried (18-23% protein)	92.1	20.7	68.2	15.5	0.16	0.73	0.47	2.13
Citrus pulp	90.5	6.5	70.5	2.0	2.08	9.43	0.11	0.50
Corn yellow, No. 2 equivalent	85.2	9.4	80.6	7.1	0.01	0.05	0.25	1.14	2.2
Corn gluten meal	92.0	43.0	78.4	37.8	0.10	0.45	0.47	2.13
Cottonseed meal (38-43% protein)	92.7	41.0	73.6	33.0	0.19	0.86	1.11	5.03
Distillers' corn grains, dried	93.2	28.1	81.6	20.1	0.04	0.18	0.29	1.32
Linseed meal (33-38% protein)	91.5	35.3	76.0	29.7	0.36	1.63	0.84	3.81
Molasses, cane	74.1	2.8	56.6	0.9	0.56	2.54	0.06	0.27
Oats	92.3	12.5	72.3	9.1	0.10	0.45	0.40	1.81
Peanut oil meal (38-43% protein)	93.6	41.6	78.8	37.0	0.10	0.45	0.50	2.27
Soybean oil meal (hyd. or exp.)	92.2	41.7	77.4	37.5	0.29	1.32	0.67	3.04
Wheat	89.4	12.0	78.6	9.1	0.05	0.23	0.38	1.72
Wheat bran	90.6	16.4	63.3	13.1	0.10	0.45	0.14	5.17

CHEMICAL COMPOSITION OF SELECTED HOG FEEDS

Feedstuff	Energy and Protein per Pound Feedstuff		Inorganic Nutrients per Pound Feedstuff				Vitamins per Pound Feedstuff						
	Total Digestible Nutrients, lb	Crude Protein, lb	Calcium, gm	Phosphorus, gm	Sodium, gm	Potassium, gm	Carotene, mg	Vitamin D, I.U.	Thiamine, mg	Riboflavin, mg	Niacin, mg	Pantothenic Acid, mg	Pyridoxine, mg
Grain													
Barley	0.72	0.13	0.32	1.45	0.26	2.32	0.19	2.71	0.55	30.44	2.84
Yellow corn	0.80	0.09	0.05	1.18	0.13	1.45	2.20	2.06	0.60	6.40	3.36	2.85
Rye	0.80	0.11	0.18	1.68	0.23	2.45	0.04	2.00	0.71	8.22	4.72
Wheat	0.74	0.12	0.23	1.68	0.14	2.36	1.15	2.10	0.51	26.74	5.62	2.07
Mill concentrates													
Rice bran	0.66	0.13	0.45	8.44	10.32	1.38	129.10	10.33	14.56
Wheat bran	0.58	0.16	0.45	5.20	0.18	5.08	1.18	3.24	1.34	139.97	11.33
Wheat middlings	0.59	0.17	0.41	4.04	0.45	4.49	1.39	7.00	0.74	52.80	7.10
Protein supplements (plant)													
Cottonseed meal (38-43%)	0.70	0.41	0.86	5.04	0.18	6.63	0.09	6.13	4.08	20.40	6.35
Linseed meal (33-38%)	0.72	0.35	1.63	3.81	0.45	5.68	0.12	5.84	2.75	22.25	3.20
Soybean meal (38-43%)	0.77	0.42	1.32	3.04	0.77	8.43	0.10	2.62	1.87	17.60	6.27
Protein supplements (animal)													
Fish meal (65%)	0.64	0.65	19.42	12.24	7.52	4.00
Meat scraps (55%)	0.80	0.55	36.15	17.52	2.00	5.22	0.55	2.78	3.54
Tankage (60%)	0.70	0.62	32.05	16.89	7.54	2.50	0.80	30.40	1.00
Skim milk (fluid)	0.08	0.03	0.59	0.41	0.23	1.09	0.21	0.85	0.47	1.63	0.73

CHEMICAL COMPOSITION OF SELECTED POULTRY FEEDS

	Mois- ture, %	Pro- tein, %	Fat, %	Fiber, %	N- Free Ex- tract, %	Ash, %	Cal- cium, %	Phos- pho- rus, %	Iron, %	Man- ga- nese, mg/lb	Cop- per, mg/lb	Co- balt, mg/lb	Caro- tene, mg/lb	Thi- amine, mg/lb	Ni- acin, mg/lb	Ribo- flavin, mg/lb	Panto- thenic Acid, mg/lb
Alfalfa meal, sun cured, 17% protein	7.8	17.6	2.0	23.8	38.9	9.9	24 *	8.8
Alfalfa meal, dehydrated, 17% protein	6.7	17.8	2.8	24.2	39.7	8.8	1.70	0.22	0.0329	15.0	3.1	0.05	36 *	1.54	8.7	7.3	12.3
Alfalfa meal, sun cured, 20% protein	8.1	20.3	3.2	17.8	40.3	10.3	48 *	7.2
Alfalfa meal, dehydrated, 20% protein	7.1	20.9	2.9	19.8	38.2	11.1	1.66	0.31	0.0385	28.6	7.1	0.17	60 *	3.10	17.3	7.4	18.5
Barley, excluding Pacific Coast	10.6	12.7	1.9	5.4	66.6	2.8	0.09	0.47	0.0049	8.3	5.1	0.01	1.7	24.1	0.8	3.7
Barley, Pacific Coast	11.0	9.7	2.2	6.2	68.7	2.2	0.06	0.41	0.0068	7.8	5.0	1.8	20.0	0.6	3.3
Bone meal, raw	6.4	26.0	5.0	1.0	2.5	59.1	21.72	10.01	0.0443	1.9	8.5	0.05	0.10	1.9	0.5	1.0
Bone meal, steamed special	3.3	13.4	9.7	1.1	1.2	71.3	29.30	15.10	0.0801	5.1	9.0	0.03	0.9	2.0	0.4	0.8
Brewers' dried yeast	6.3	46.8	1.2	2.8	35.7	7.2	0.11	1.52	0.0138	2.4	15.1	0.08	43.0	213.6	14.0	49.1
Buttermilk, dried	7.6	32.4	6.4	0.3	43.3	10.0	1.35	0.94	1.5	1.7	2.8	15.8	13.5
Corn, dent, yellow	15.0	8.9	3.9	2.0	68.9	1.3	0.023	0.27	0.002	2.3	0.9	0.01	1.33	1.7	9.8	0.5	2.6
Corn gluten meal, 41% protein	8.6	42.9	2.0	3.9	40.1	2.5	0.20	0.41	0.0467	4.4	13.1	0.04	10.0	24.8	0.7	3.8
Cottonseed meal, 41% protein	7.8	41.2	6.2	11.2	27.7	5.9	0.180	1.14	0.0076	12.9	7.7	0.06	1.8	13.0	2.5	4.4
Crab meal	7.9	31.5	2.2	10.5	6.0	41.9	14.50	1.50	2.3	3.0
Distillers' dried corn grains, with solubles	6.9	28.8	8.9	9.0	41.7	4.7	0.16	0.74	18.2	2.1	36.3	3.4	5.2
Distillers' dried solubles	6.9	29.0	6.7	3.3	47.6	7.5	0.35	1.40	0.0500	45.4	36.3	0.09	2.7	54.3	5.2	8.9
Fish meal, menhaden	6.4	62.2	8.5	0.7	4.2	18.0	5.00	3.40	0.0570	10.0	3.9	0.08	0.2	25.9	2.4
Fish meal, sardine	6.9	67.2	5.0	0.6	5.4	14.9	4.21	2.54	0.03	10.3	9.2	0.08	2.5
Fish meal, whitefish	9.6	63.0	6.7	0.1	0.1	20.5	6.76	3.69	5.0	0.4	36.0	4.0
Hominy feed, white, 5% fat	10.1	10.8	5.7	4.7	66.0	2.7	0.05	1.03	0.0039	8.2	9.9	5.9	25.1	1.0	3.1
Hominy feed, yellow	9.5	11.1	7.1	5.8	63.9	2.6	0.05	0.66	0.0235	6.9	5.7	0.02	4.1	3.8	22.6	1.0	3.5
Limestone	38.30
Linseed oil meal, old process, 33% protein	8.8	35.0	5.6	8.1	36.9	5.6	0.44	0.94	0.0240	19.1	11.8	0.17	3.9	18.9	1.9	7.5

Linseed oil meal, old process, 37% protein	9.1	38.0	5.9	7.7	33.7	5.6	0.49	0.89	0.0276	18.6	11.9	0.18	2.7	14.7	1.7	8.9
Meat scrap, 52% protein	6.9	52.9	7.3	2.2	4.3	26.4	8.7	4.40	0.0503	4.0	5.5	0.09	0.03	27.1	2.4	2.1
Meat scrap, 60% protein	6.2	60.9	8.8	2.4	1.1	20.6	6.3	3.50	0.0410	5.4	3.7	0.04	0.10	23.7	2.5	2.3
Meat and bone scrap, 50% protein	6.4	50.6	10.0	2.0	2.0	29.0	9.7	4.2	5.3	21.4	2.1	1.5
Molasses, beet	19.5	8.4	62.0	10.1	0.08	0.02	22.0
Molasses, cane	26.0	2.9	62.1	9.0	0.74	0.08	0.4	20.9	1.1	17.9
Oats, excluding Pacific Coast	9.8	12.0	4.6	11.0	58.6	4.0	0.09	0.43	0.0080	19.2	2.4	2.9	8.2	0.4	6.8
Oats, Pacific Coast	9.8	9.0	5.4	11.0	61.1	3.7
Oatmeal, feeding	9.3	15.0	7.4	2.0	64.4	1.9	0.07	0.43	0.0065	13.6	1.6	0.02	3.5	4.5	0.6	6.6
Oyster shell, ground	37.9
Peanut oil meal, old process, 43% protein	7.2	43.1	7.6	13.9	23.0	5.2	0.16	0.54	3.3	77.5	2.4	24.1
Phosphate, defluorinated rock	28.3	12.3	40.90	29.5
Phosphate, dicalcium	26.5	20.5
Rice bran	9.0	12.8	13.1	12.7	41.7	10.7	0.08	1.36	70.9
Rice polishings	9.7	12.7	11.4	3.5	56.6	6.2	0.04	1.10	10.3	129.1	1.4	10.3
Rye	10.5	12.6	1.7	2.4	70.9	1.9	0.01	0.33	0.008	37.0	3.4	8.8	325	0.9	5.5
Skim milk, dried	5.8	34.7	1.2	0.2	50.3	7.8	1.27	1.10	0.0054	1.2	5.2	0.03	2.0	7.1	0.7	4.2
Sorghum, kafir	10.5	11.2	2.9	2.3	71.1	2.0	0.04	0.330	0.0084	7.5	3.0	0.03	1.5	5.7	10.0	16.0
Sorghum, milo	10.6	11.3	2.9	2.2	71.3	1.7	0.03	0.27	0.0053	5.9	7.8	0.02	1.6	18.3	0.5	5.7
Sorghum, milo head chop	10.0	10.1	2.5	6.8	67.1	3.5	0.14	0.26	1.8	13.1	0.4	5.0
Soybean oil meal, 41% protein	10.2	41.1	5.3	5.5	30.4	7.5	0.26	0.59	0.017	12.3	11.1	0.9
Soybean oil meal, 43% protein	9.4	43.1	5.6	5.7	30.4	5.8	0.28	0.61	12.7	2.5
Soybean oil meal, 44% protein	9.2	44.2	5.3	5.6	29.9	5.8	0.30	0.66	0.015	14.0	7.7	0.05	1.5
Soybean oil meal, solvent extracted	9.4	46.1	1.0	5.9	31.8	5.8	0.29	0.63	0.0100	13.8	6.8	0.8	16.7	2.0	6.1
Wheat, hard red, winter	10.4	15.2	1.8	2.6	68.3	1.7	0.05	0.41	0.0053	18.0	2.0	0.03	1.4	17.1	1.4	6.2
Wheat, northern, spring	9.9	15.8	2.2	2.5	67.8	1.8	0.05	0.41	0.0050	33.0	4.9	2.3	24.1	0.5	6.3
Wheat, soft, Pacific Coast	10.8	9.9	2.0	2.7	72.7	1.9	0.29	0.0069	27.7	4.4	2.3	28.8	0.5	6.4
Wheat bran	10.3	16.4	4.3	9.9	53.0	6.1	0.14	1.30	0.0162	56.0	4.9	0.05	2.2	26.8	0.5	5.2
Wheat flour middlings	10.3	18.1	4.6	4.9	58.5	3.6	0.07	0.65	0.0022	39.0	2.1	3.9	63.5	1.4	13.6
Wheat standard middlings	9.9	17.6	5.0	6.7	56.6	4.2	0.14	0.78	0.0090	53.6	9.8	0.04	6.0	44.2	0.8	4.5
Whey, dried	6.5	12.2	0.8	0.2	70.4	9.9	0.91	0.75	0.0212	1.1	24.2	0.05	5.8	44.3	0.8	9.3
														1.8	5.1	13.0	22.4

* Rough approximations, since carotene content is too variable for dependable averages.

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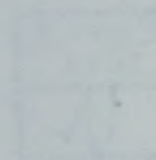
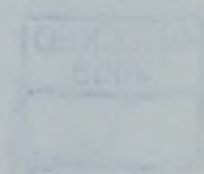
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